

Historical trends of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in fish and sediment associated with two bleached kraft pulp mills in northern Ontario

By

Shari Cater

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Science

in

Biology

Waterloo, Ontario, Canada, 2013

©Shari Cater 2013

Author's Declaration

I hereby declare that I am the sole author of this thesis except where noted. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

In the early 1990s polychlorinated dibenzo-*p*-dioxin (PCDD) and dibenzofuran (PCDF) contamination of fish was widely associated with bleached kraft pulp mills. Regulations were put into place in Canada and elsewhere to reduce or eliminate the presence of these chemicals in pulp mill effluents. The industry quickly introduced treatment and process changes such as elemental (ECF) and total chlorine free (TCF) bleaching, which resulted in dramatically reduced PCDD/F concentrations in pulp mill effluents. However, PCDD/Fs may remain a concern for the receiving environment near bleached kraft pulp mills due to their tendency to persist in sediments and bioaccumulate in aquatic biota.

Several studies conducted in the early 1990s reported significantly elevated levels of PCDD/Fs in white sucker (*Catostomus commersoni*) exposed to bleached kraft mill effluent (BKME). Particularly high concentrations were observed in the receiving environment of two mills in northern Ontario. The first mill, located in the town of Terrace Bay, discharges effluent into Jackfish Bay, Lake Superior. The second mill is located in Smooth Rock Falls, Ontario and discharges effluent into the Mattagami River where effects have been observed in fish collected downstream of the pulp mill. Over time, both mills have undergone a number of process upgrades, including a transition to 100% chlorine dioxide (ClO₂) substitution. In 2006 the Tembec Smooth Rock Falls Pulp and Paper Mill closed permanently, while the mill in Terrace Bay has gone through a number of temporary shutdowns and is currently in the process of transitioning to a dissolving pulp process.

Temporal changes in PCDD/F contamination was examined in white sucker historically exposed to BKME in Jackfish Bay and the Mattagami River, following mill process changes and

closures. Historical data was summarized from studies conducted in the 1990s and analyzed along with liver tissue of male white sucker collected from each location in 2011 and 2012. The body burden of each fish was analysed using Toxic Equivalency (TEQ) calculations to account for concentration and relative toxicity of 2,3,7,8– substituted PCDD/F congeners. At Jackfish Bay, concentrations of PCDD/Fs in surface sediment and dated sediment cores were used to document the spatial and temporal pattern of PCDD/F contamination in relation to historical process upgrades and operational changes at the mill.

PCDD/Fs measured in white sucker liver samples collected from Jackfish Bay during gonadal recrudescence in the fall illustrate a decrease in mean TEQ from $74.2 \pm 20.9 \text{ pg}\cdot\text{g}^{-1}$ in 1991 to $3.34 \pm 2.05 \text{ pg}\cdot\text{g}^{-1}$ in 2012. These values were slightly elevated compared to the remote reference location at Mountain Bay, Lake Superior, which exhibited a mean TEQ of $1.88 \pm 0.45 \text{ pg}\cdot\text{g}^{-1}$ and $1.06 \pm 0.69 \text{ pg}\cdot\text{g}^{-1}$ in white sucker collected in fall 2011 and 2012, respectively. Although below consumption guidelines, trace levels of PCDD/Fs persist in fish collected from Jackfish Bay and these concentrations are suspected to reflect sediment contamination in Moberly Bay (part of Jackfish Bay). A unique PCDD/F contaminant profile, dominated by 2,3,7,8–tetrachlorodibenzo-*p*-dioxin (TCDD) and dibenzofuran (TCDF), was observed in white sucker and sediment collected from Jackfish Bay. Analysis of surface sediment collected throughout Jackfish Bay revealed elevated PCDD/F concentrations, compared to reference areas in Lake Superior. TEQ values measured in surface sediment from the depositional areas of Moberly Bay exceeded the Canadian Council of Ministers of the Environment (CCME) guidelines.

In 1991 PCDD/F contamination, reported as mean TEQ, of white sucker collected downstream of the pulp mill outfall in Smooth Rock Falls reached levels of $111 \pm 86.2 \text{ pg}\cdot\text{g}^{-1}$. At

the time, the mill utilized molecular chlorine (Cl_2) in the bleaching sequence and employed primary effluent treatment. A dramatic decline was observed in PCDD/F concentrations of fish collected in 1993 and 1995, corresponding to implementation of 100% ClO_2 substitution in the bleaching process. PCDD/F contamination in white sucker collected from the Mattagami River in 2011 and 2012 were similar upstream ($0.53\text{--}1.49\text{ pg TEQ}\cdot\text{g}^{-1}$) and downstream ($0.75\text{--}2.87\text{ pg TEQ}\cdot\text{g}^{-1}$) of the historical pulp mill outfall at Smooth Rock Falls, suggesting a return to background condition following the 2006 mill closure. Levels of 2,3,7,8-TCDD and 2,3,7,8-TCDF in liver tissue of fish collected downstream declined drastically compared to concentrations measured in 1991. The PCDD/F congener profile observed in white sucker downstream in 2011 and 2012 was more similar to fish collected from the reference site upstream than exposed fish collected in 1991. No sediment depositional areas suitable for taking a core sample were found on the river downstream of Smooth Rock Falls due to the nature of the system.

This study supports the conclusion that particulate (POM) and dissolved organic matter (DOM) from continuous inputs of effluent were likely the primary source of PCDD/Fs to fish in these receiving environments, with only a small contribution from surface sediment. Results indicate a decreasing trend in PCDD/F contamination of white sucker historically exposed to bleached kraft pulp mill effluent in the receiving environments of Jackfish Bay and the Mattagami River, consistent with mill process upgrades to eliminate the use of elemental chlorine from the bleaching sequence. Current levels of PCDD/Fs measured in white sucker collected from these locations suggest a return to background condition.

Acknowledgements

Much gratitude is due to my supervisor, Dr. Mark Servos, for his guidance and encouragement over the past two years and for his hands-on involvement in the project. I am grateful for his tireless effort in helping me find the perfect sediment cores. I would also like to thank my committee members, Dr. Mark McMaster and Dr. George Dixon, for their valuable input. A special thanks to Kelly Munkittrick for his involvement in this project. I would like to extend my thanks to Roland Hall for allowing me access to his lab to prepare my sediment samples and to Johan Wiklund for performing core chronology analysis on my sediment cores.

I am grateful to all members of the Servos lab, past and present, for their support and friendship. I feel incredibly lucky to have had the opportunity to work with and learn from everyone. I would especially like to thank Ken Oakes and Lisa Bowron for the mountain of journal articles and reports they shared with me. Thank you, for the time and effort you put into tracking those down and for saving them for many years.

I could not have completed my field work without the help of a number of people: Jessica Mendoza, Lisa Bowron, Gerald Tetreault, Jim Bennett, Maria Villella, Patricija Marjan, Meghan Fuzzen, Jen Ings, Paulina Bahamonde, Cam Portt. I am eternally grateful for your hard work and positive attitude despite long, cold days in the field. Thanks are due to Tim Arciszewski for organizing fish sampling from Smooth Rock Falls and for accommodating my requests for data.

Finally, I wish to thank my friends and family, especially Mark Dahmer, for their support and patience. I could not have done this without you.

Thank you!

Table of Contents

Author's Declaration.....	ii
Abstract.....	iii
Acknowledgements.....	vi
List of Figures.....	ix
List of Tables.....	x
Chapter 1 General Introduction.....	1
1.0 Introduction.....	1
1.1 Pulp Mills.....	1
1.1.1 Types of Pulp Mills.....	1
1.1.2 Bleaching.....	2
1.2 Environmental Monitoring.....	5
1.3 Polychlorinated Dibenzo- <i>p</i> -Dioxins and Furans (PCDD/Fs).....	6
1.3.1 Fate and Distribution.....	7
1.3.2 Mechanism of Action.....	10
1.3.3 Toxic Effects of PCDD/Fs.....	12
1.3.4 Toxic Equivalency (TEQ).....	12
1.4 Study sites.....	13
1.4.1 Jackfish Bay.....	13
1.4.2 Smooth Rock Falls.....	21
1.5 Overview of evidence for environmental impact of bleached kraft mill effluent in two northern Ontario mills.....	25
1.5.1 Fish.....	25
1.5.2 Sediment.....	26
1.6 Objectives.....	27
Chapter 2 Historical trends of polychlorinated dibenzo- <i>p</i> -dioxins and dibenzofurans in white sucker (<i>Catostomus commersoni</i>) liver and dated sediment cores from Jackfish Bay, Lake Superior.....	29
2.1 Summary.....	30
2.2 Introduction.....	31
2.3 Materials and Methods.....	34

2.3.1	Study site.....	34
2.3.2	Fish collection and sampling procedures	35
2.3.3	Sediment Collection.....	36
2.3.4	²¹⁰ Pb Dating of Sediment Cores.....	36
2.3.5	PCDD/F analysis.....	37
2.3.6	Statistical analysis	38
2.4	Results	40
2.4.1	White sucker	40
2.4.2	Sediment	48
2.5	Discussion	55
Chapter 3 Historical trends of polychlorinated dibenzo- <i>p</i> -dioxins and dibenzofurans in white sucker (<i>Catostomus commersoni</i>) from the Mattagami River, following closure of the bleached kraft pulp mill at Smooth Rock Falls		66
3.1	Summary	67
3.2	Introduction	68
3.3	Materials and Methods	70
3.3.1	Study site.....	70
3.3.2	Fish collection and sampling procedures	71
3.3.3	PCDD/F analysis.....	72
3.3.4	Statistical analysis	72
3.4	Results	72
3.5	Discussion	79
Chapter 4 General Conclusions		83
References		86
Appendix A Supporting information for Chapter 2.....		100
Appendix B Supporting information for Chapter 3		110

List of Figures

Figure 1.1 The general structure of polychlorinated dibenzo- <i>p</i> -dioxin (PCDD) and dibenzofuran (PCDF) (Fletcher and McKay 1993)	7
Figure 1.2 Fate and distribution model for PCDD/Fs in aquatic environments.	9
Figure 1.3 Mode of action of PCDD/Fs in the cell	11
Figure 1.4 Map of Jackfish Bay Area of Concern (AOC)	16
Figure 1.5 Map of Moose River basin	22
Figure 2.1 TEQ ($\text{pg}\cdot\text{g}^{-1}$ wet wt.) and lipid normalized TEQ ($\text{pg}\cdot\text{g}^{-1}$ lipid) for liver tissue of male white sucker collected from Jackfish Bay (black) and Mountain Bay (grey) during the fall.....	43
Figure 2.2 TEQ ($\text{pg}\cdot\text{g}^{-1}$ wet wt.) and lipid normalized TEQ ($\text{pg}\cdot\text{g}^{-1}$ lipid) for liver tissue of male white sucker collected from Jackfish Bay and Mountain Bay during spring	46
Figure 2.3 TEQ ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and organic matter (OM) normalized TEQ ($\text{pg}\cdot\text{g}^{-1}$ OM) for a dated sediment core collected from Moberly Bay.	50
Figure 2.4 TEQ ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and organic matter (OM) normalized TEQ ($\text{pg}\cdot\text{g}^{-1}$ OM) for dated sediment cores collected from Moberly Bay, Jackfish Bay and Santoy Bay.	51
Figure 2.5 TEQ ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and organic matter normalized TEQ ($\text{pg}\cdot\text{g}^{-1}$ OM) for surface sediment collected at various distances from Blackbird Creek.	54
Figure 3.1 TEQ ($\text{pg}\cdot\text{g}^{-1}$ wet wt.) and lipid normalized TEQ ($\text{pg}\cdot\text{g}^{-1}$ lipid) for liver tissue of male white sucker collected upstream and downstream of the pulp mill at Smooth Rock Falls	77
Figure 3.2 TEQ ($\text{pg}\cdot\text{g}^{-1}$ wet wt.) and lipid normalized TEQ ($\text{pg}\cdot\text{g}^{-1}$ lipid) for liver tissue of male white sucker collected upstream and downstream of the pulp mill at Smooth Rock Falls	78
Figure A.1 Sampling sites for surface sediment and sediment cores collected throughout Jackfish Bay and Santoy Bay.	1042
Figure A.2 Activity profile of sediment core collected from Moberly Bay	104
Figure A.3 Age depth profiles (CRS and CF:CS models) for Moberly Bay sediment core.....	105
Figure A.4 ^{137}Cs activity vs. CRS age for Moberly Bay sediment core	106
Figure A.5 CRS determined dry mass sedimentation rates vs. CRS age for Moberly Bay sediment core	106

List of Tables

Table 1.1 Toxic equivalency factors (TEF) of PCDD/F congeners for mammals and fish reported by the World Health Organization (WHO).....	13
Table 1.2 Summary of process changes at the bleached kraft pulp mill in Terrace Bay, ON	17
Table 1.3 Summary of process changes at the bleached kraft pulp mill in Smooth Rock Falls, ON.....	23
Table 2.1 PCDD/F concentrations ($\text{pg}\cdot\text{g}^{-1}$, mean \pm SD) with wet weight ($\text{pg}\cdot\text{g}^{-1}$ wet wt., mean \pm SD) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ lipid, mean \pm SD)) in liver tissue of male white sucker collected from Jackfish Bay and Mountain Bay during the fall.....	41
Table 2.2 PCDD/F concentrations ($\text{pg}\cdot\text{g}^{-1}$, mean \pm SD) with wet weight ($\text{pg}\cdot\text{g}^{-1}$ wet wt., mean \pm SD) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ lipid, mean \pm SD) TEQ in liver tissue of male white sucker collected from Jackfish Bay and Mountain Bay during the spring.....	44
Table 2.3 TEQ ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and organic matter (OM) normalized TEQ ($\text{pg}\cdot\text{g}^{-1}$ OM) for surface sediment collected from various distances from Blackbird Creek.....	53
Table 3.1 PCDD/F concentrations ($\text{pg}\cdot\text{g}^{-1}$, mean \pm SD) with wet weight ($\text{pg}\cdot\text{g}^{-1}$ wet wt., mean \pm SD) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ lipid, mean \pm SD) in liver tissue of male white sucker collected in the fall upstream (MAT-UP) and 1 km (MAT-DN) and 30 km (MAT-CY) downstream of the pulp mill at Smooth Rock Falls	75
Table A.1 Sediment sampling sites, sample ID, type of sample, GPS coordinates and distance from Blackbird Creek (BBC).....	101
Table A.2 PCDD/F concentration ($\text{pg}\cdot\text{g}^{-1}$), wet weight ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ OM) TEQ for dated sediment core collected in Moberly Bay.	107
Table A.3 PCDD/F concentration ($\text{pg}\cdot\text{g}^{-1}$), wet weight ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ OM) TEQ for dated sediment core collected in Santoy Bay.....	108
Table A.4 PCDD/F concentration ($\text{pg}\cdot\text{g}^{-1}$), wet weight ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ OM) TEQ for dated sediment core collected in Jackfish Bay near St. Patrick Island	109
Table B.1 International toxic equivalency factors (I-TEF) of PCDD/F congeners for mammals	110

Chapter 1 General Introduction

1.0 Introduction

The pulp and paper industry has historically been one of the largest employers in Canada and has allowed Canada to be a significant exporter of newsprint and pulp to the world market. Pulp and paper mills have been identified as major contributors of point and non-point source pollution. Atmospheric deposition and spill events provide a source of contamination; however, wastewater effluent has historically been the primary concern.

1.1 Pulp Mills

Pulp and paper products are primarily composed of wood, the constituents of which are cellulose, hemicellulose, lignin and wood extractives. Wood undergoes debarking and chipping to produce raw wood chips for use in pulping (Bajpai 2012). During pulp production wood chips undergo pulping, washing, bleaching and extraction to obtain the cellulose fibers required to produce the final product. The pulping process separates and recovers cellulose fibers from lignin and other wood components through various combinations of mechanical, thermal and chemical process treatments (Bajpai 2012).

1.1.1 Types of Pulp Mills

The major pulping processes include mechanical, sulphite and sulphate (kraft). Mechanical pulping involves shredding or grinding wood through an energy intensive process and is capable of converting up to 95% of the dry weight of wood into pulp (Smook 1989). The pulp produced from this process is commonly used as the major component in newsprint due to

its good printing properties; however, it produces a weaker paper product compared to chemical pulps (Bajpai 2012). Sulphite pulping uses a cooking liquor composed of sulphurous acid (H_2SO_3) and calcium, magnesium, ammonium or sodium bisulphite (HSO_3^-) to obtain cellulose fibers (Smook 1992). Sulphite pulping is well suited for certain species of tree, and produces a light coloured pulp that can be easily bleached. The kraft process, however, produces a superior product in terms of strength and chemical recovery (Bajpai 2012).

The term 'kraft', of German origin, refers to the strength of the end product. Kraft pulp is produced by digesting wood chips under high temperature and pressure conditions with an alkaline cooking liquor composed of sodium hydroxide (NaOH) and sodium sulphite (NaS_2) to dissolve lignin and allow the wood fibers to separate (Smook 1989). Kraft processing is able to maintain pulp strength by ensuring an optimal cooking temperature of $155\text{--}175^\circ\text{C}$ is achieved and that the cooking process is terminated at a point where a suitable level of residual lignin remains (measured by the Kappa number) (Smook 1992). Although this results in a lower yield, darker color and higher residual lignin content (lower kappa number) of pulp compared to other pulping processes, the advantages of kraft pulping have allowed it to become the dominant pulping practice worldwide (Bajpai 2012).

1.1.2 Bleaching

Cellulose and hemicellulose are naturally white, while chromophoric groups on lignin tend to absorb light, therefore lowering the brightness level of kraft pulp (Smook 1992). At bleached kraft mills, additional lignin has traditionally been removed using hypochlorite (ClO^-), chlorine gas (Cl_2) or chlorine dioxide (ClO_2) to allow the desired degree of brightness to be reached for a variety of paper products. Bleaching processes often involve multiple stages of

delignification and extraction to produce the desired level of brightness and remove by-products. A conventional five stage bleaching sequence may include the following stages: chlorination (C), alkaline extraction (E), chlorine dioxide (D), alkaline extraction (E), chlorine dioxide (D) and can be designated as CEDED (Smook 1992). Each stage is followed by washing to remove reaction products and the bleaching stages alternate between acidic and alkaline to reduce the formation of reaction products. The alkaline extraction stage serves to further de-lignify the pulp by removing chlorinated and oxidized lignin via solubilisation following the initial chlorination (Bajpai 2013).

This process has evolved over the years as the pulp and paper industry has made process changes to increase efficiency and decrease environmental effects. The use of Cl_2 in bleaching produces chlorinated organic compounds such as resin acids, chlorinated phenolics and polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). The amount of chlorinated organic contaminants produced by the bleaching process varies based on the wood species used, lignin content and bleaching sequence used by each mill (Axegård and Renberg 1989, Bajpai 2013). Effluents from mills using elemental chlorine in their bleaching sequence have been associated with adverse effects on aquatic environments (McMaster *et al.* 1991, Munkittrick *et al.* 1991, Munkittrick *et al.* 1994, Sandstrom *et al.* 1988, Södergren 1989). The formation of PCDD/Fs has been attributed primarily to direct chlorination of precursors, such as dibenzodioxin (DBD) and dibenzofuran (DBF), present in brown stock washers (Allen *et al.* 1989, Berry *et al.* 1991, Luthe and Berry 1996, Voss *et al.* 1988). Additional PCDD/F congeners can also be formed during the E stage of the bleaching process (LaFleur *et al.* 1990). LaFleur *et al.* (1990) illustrated equal formation of 2,3,7,8- tetrachlorodibenzo-*p*-dioxin (TCDD) from the

C and E stages, while majority of the 2,3,7,8-tetrachlorodibenzofuran (TCDF) congener was formed by the C stage.

When the industry moved toward elemental chlorine free (ECF) bleaching practices, such as the use of ClO_2 , PCDD/Fs from mill effluents were greatly reduced (Luthe *et al.* 1992, Servos *et al.* 1997, Yunker *et al.* 2002). Although ECF bleaching can produce Cl_2 as an intermediate, the concentrations were significantly lower compared to those used in elemental chlorine bleaching practices (Smook 1992). Upgrades to the bleaching process, through the use of the R8[®] ClO_2 generation process, later eliminated production of chlorine by-products (Smook 1992). A reduction of water consumption was achieved through an increase in the use of recycled effluent and subsequent decrease in brown stock consumption. This led to an increase in the process temperature and the potential to affect by-product formation (Smook 1992).

Pulp and paper mills have since made further changes to their manufacturing practices through the use of total chlorine free (TCF) bleaching, including the use of hydrogen peroxide (H_2O_2) and oxygen (O_2) to brighten products. Extended delignification and oxygen delignification are typically used as a pre-bleaching step because of their ability to reduce lignin content by up to 50% while preserving cellulose (Bajpai 2013). Hydrogen peroxide has been used in varying capacities in the pulp and paper industry for over 60 years. The use of hydrogen peroxide as the primary bleaching agent at bleached kraft mills, however, did not occur until much later (Larisch and Duff 1997) and it is most efficient when utilized during the second stage of the bleaching sequence (Bajpai 2013). Although it offers numerous advantages to traditional bleaching, the majority of mills were slow to convert to TCF processes due to the resulting lower quality pulp and the reduced product strength, brightness and yield (Smook 1992).

1.2 Environmental Monitoring

In the late 1980s the results of Swedish studies investigating the impact of pulp and paper mills to aquatic environments suggested that chronic effects were occurring in fish exposed to bleached pulp mill effluent (Sandstrom *et al.* 1988, Södergren 1989). In response to these results, similar studies were initiated at pulp mills across Canada (McMaster *et al.* 1991, Munkittrick *et al.* 1991, Munkittrick *et al.* 1994). Early findings in Canada supported the conclusions of studies conducted in Sweden, including reproductive effects observed in fish exposed to low effluent concentrations (reviewed by McMaster *et al.* 2006, Munkittrick *et al.* 2013, Walker *et al.* 2002).

As results of the potential impact of bleached kraft mill effluents on aquatic ecosystems were being published, the Canadian and U.S governments were considering modifications to regulations imposed on effluents from pulp and paper mills. In the 1970s and 1980s discharges from Canadian pulp and paper mills were controlled federally by the Pulp and Paper Effluent Regulations (PPER). Although these regulations addressed acute toxicity of effluents, limited biological oxygen demand (BOD) and total suspended solids (TSS) they did not address discharges of chlorinated organic compounds or chronic effects on aquatic biota (CEPA 1999). Despite global pressure to regulate the use of molecular chlorine in pulp and paper manufacturing due to their persistence and demonstrated toxicity in laboratory settings, the role that chlorinated contaminants might play in causing the observed effects in fish residing in bleached kraft mill effluent (BKME) receiving environments remained unknown. Evidence was beginning to emerge, however, to suggest there would still be chronic effects observed in fish following compliance with regulations to reduce the formation of chlorinated organic compounds (Hodson *et al.* 1992, Servos *et al.* 1992a). In 1992 the Canadian Environmental Protection Act (CEPA) implemented regulations on the discharge of PCDD/Fs from pulp and

paper mills, requiring a reduction to non-measurable levels (CEPA 1999). Updates to the PPER were legally binding at all Canadian mills and included the additional requirement that Environmental Effects Monitoring (EEM) be conducted at all sites (CEPA 1999).

The industry-funded pulp and paper EEM program was designed to provide information to the government to help determine the proportion of receiving environments associated with environmental effects following the compliance of mills with effluent regulations (Environment Canada 1998). The specific objective of EEM is to assess the effectiveness of regulations in protecting fish, fish habitat and use of fisheries resources. Results of the first cycle EEM studies at Canadian mills were reported to Environment Canada by April 1996 and the second cycle was initiated in 1997. Subsequent cycles of EEM monitoring have occurred every three years and utilize a standard decision tree used to aid with interpretation of the results. The first cycle required all mills to report effluent concentrations of PCDD/Fs. During this time the industry was transitioning toward the use of ECF bleaching processes to comply with new effluent regulations. This resulted in the virtual elimination of PCDD/Fs from mill eluent at the majority of mills and measurement of PCDD/Fs were removed from subsequent cycles of EEM.

1.3 Polychlorinated Dibenzo-*p*-Dioxins and Furans (PCDD/Fs)

In the mid-1980s international concern arose regarding the presence of PCDD/Fs, which are well known environmental contaminants, in bleached kraft pulp mill effluent and paper products. The extent and position of Cl substitution can produce up to 75 PCDD and 135 PCDF congeners, including: tetra (T), penta (Pe), hexa (Hx), hepta (Hp) and octa (O) –chlorodibenzo-*p*-dioxins and furans (Fig. 1.1) (Liu 2007, Poland and Knutson 1982, Walker et al. 2006). PCDD/Fs are stable compounds with high melting points, low vapour pressure, high octanol-

water partitioning coefficients (K_{ow}) and very low water solubility, which decrease with an increase in Cl substitution of congeners (Friesen et al. 1990, Shiu et al. 1988). These physical and chemical properties play an important role in the fate and distribution of PCDD/Fs in the environment. Highly chlorinated PCDD/Fs tend to persist in sediments and bioaccumulate in aquatic biota because they have long half-lives and are not readily degraded (Clemons *et al.* 1997, Muir and Servos 1996, Segstro *et al.* 1995).

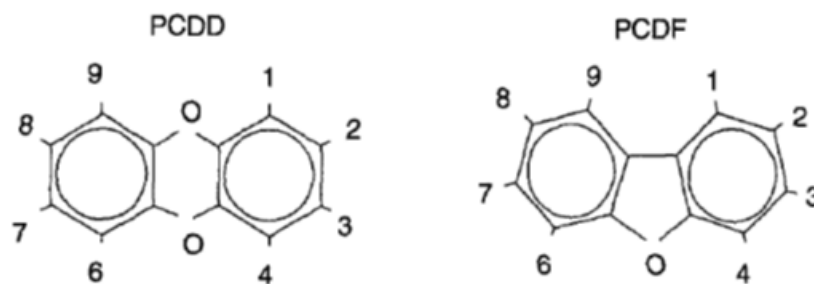


Figure 1.1 The general structure of polychlorinated dibenzo-*p*-dioxin (PCDD) and dibenzofuran (PCDF), where the numbered carbons indicate potential for chlorine substitution (Fletcher and McKay 1993).

1.3.1 Fate and Distribution

Once PCDD/Fs enter aquatic environments they follow one of three distribution pathways: dissolve in the water column, adsorb to dissolved organic matter (DOM) or associate with particulate organic matter (POM) (Fig. 1.2). Because of their nonpolar, hydrophobic nature, the majority of PCDD/Fs bind to DOM and POM in aquatic environments and become incorporated in the sediment as they settle to the bottom of the water column (Fletcher and McKay 1993, Muir *et al.* 1992, Segstro *et al.* 1995, Servos *et al.* 1989a, Servos *et al.* 1992b). The presence of highly chlorinated, superlipophilic PCDD/Fs in sediment has been well documented in the Great Lakes (Bhavsar *et al.* 2008, De Vault *et al.* 1989, Huestis *et al.* 1997, Segstro *et al.* 1995, Servos *et al.* 1992a, 1994).

In aquatic ecosystems, PCDD/Fs deposited in sediments are considered to be bioavailable for many years before they become buried or transported (Segstro *et al.* 1995). Processes such as burial, mixing, resuspension, diffusion into pore water and insect emergence can alter the bioavailability of PCDD/Fs found in sediments (Segstro *et al.* 1995). Following the addition of highly chlorinated congeners to aquatic ecosystems, PCDD/Fs become bioavailable at the sediment surface (top 2–5 cm) as it undergoes mixing processes before becoming buried (Fig. 1.2) (Servos *et al.* 1989b). A decrease in bioavailability from tetra to octa chlorinated congeners has been demonstrated in sediments and can be explained by the lower $\log K_{ow}$ and smaller size of tetrachlorodibenzo-*p*-dioxins and furans (TCDD/Fs) compared to congeners with a higher degree of chlorination (Geyer *et al.* 1992, Muir and Servos 1996, Segstro *et al.* 1995). Additionally, preferential accumulation of 2,3,7,8–substituted congeners has been observed in fish (Kuehl *et al.* 1987).

Uptake of PCDD/Fs into fish can occur directly from the dissolved phase in the water column or through ingestion of sediment as well as feeding on benthic organisms (which may ingest surface and suspended sediment), resulting in bioconcentration and bioaccumulation. Because of their high $\log K_{ow}$, the primary route of exposure of PCDD/Fs to biota is through the diet with bottom feeding and older, fatty fish and filter feeding organisms exhibiting the highest rates of bioaccumulation (Kuehl *et al.* 1987, Muir *et al.* 1992, Thomann 1989, US-EPA 1993). Once PCDD/Fs have crossed the gill or gut membrane in fish they enter into the circulatory system where they are transported to sites of storage, metabolism, excretion or action. Hydrophobic compounds such as PCDD/Fs tend to accumulate in large concentrations in tissues

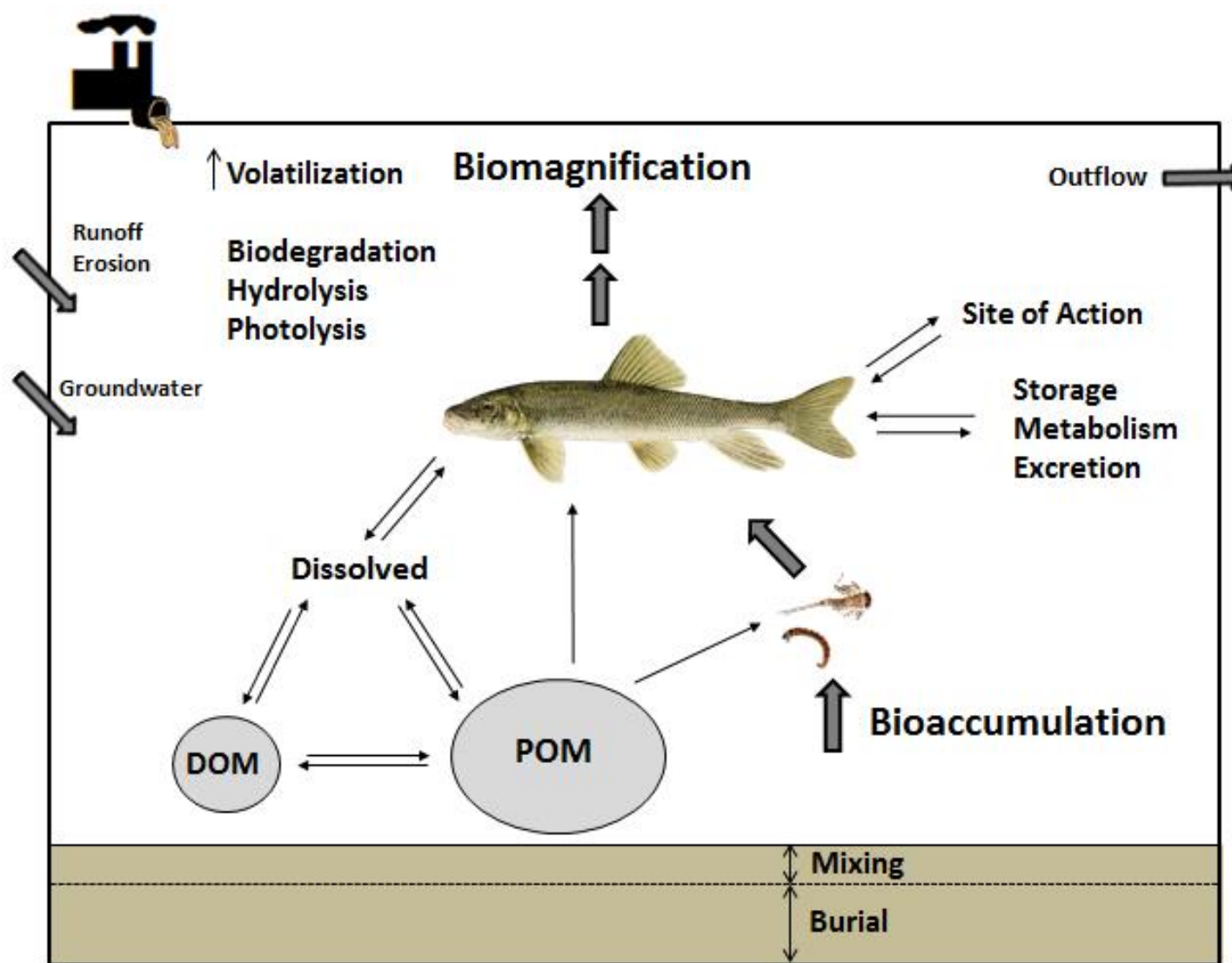


Figure 1.2 Fate and distribution model for PCDD/Fs in aquatic environments. Partitioning of PCDD/Fs from water into air, sediment and biota is exhibited along with processes affecting bioavailability. Once taken up by fish the dioxins may bioconcentrate and bioaccumulate in tissues or biotransform through metabolic reactions. Adapted from Servos *et al.* (1992b).

with high lipid content such as the liver. For this reason, measured concentrations are often normalized to the lipid content of the tissue of interest (Kuehl *et al.* 1987, Servos *et al.* 1994).

1.3.2 Mechanism of Action

Because PCDD/Fs are nonpolar and highly lipophilic they are able to diffuse across the cell membrane where they bind to the aryl hydrocarbon receptor (AhR) within the cytosol, initiating a toxic response pathway (Fig. 1.3) (Delescluse *et al.* 2000, Mandal 2005). The most toxic chlorinated organic compounds are those with a planar configuration that allows them to interact with the AhR. PCDD/F congeners with four to six chlorine substitutions possess the greatest binding potential with 2,3,7,8-TCDD having the highest binding affinity and therefore the greatest toxicity (Delescluse *et al.* 2000, Friesen *et al.* 1990, Mandal 2005).

The AhR transcription factor has been studied extensively in mammals with an increasing body of work related to the AhR in fish (Hahn 2001). The AhR is a ligand-activated transcription factor that is always present in the cytosol of the cell. Two heat shock (Hsp90) proteins act on AhR to facilitate ligand binding by maintaining the appropriate receptor conformation and stabilizing AhR (Delescluse *et al.* 2000, Mandal 2005). In fish the two Hsp90 proteins have been identified as Hsp90 α and Hsp90 β ; expression of both is induced by stressful conditions within the cell, including the presence of environmental pollutants such as PCDD/Fs (Zhou *et al.* 2010). Once a ligand has bound to the AhR, the two Hsp90 proteins dissociate and the ligand-AhR complex is translocated to the nucleus where it binds to the aryl hydrocarbon receptor nuclear translocator (ARNT) (Delescluse *et al.* 2000, Mandal 2005). This transcriptionally active complex then binds dioxin response elements (DRE) resulting in activation of transcription (Delescluse *et al.* 2000).

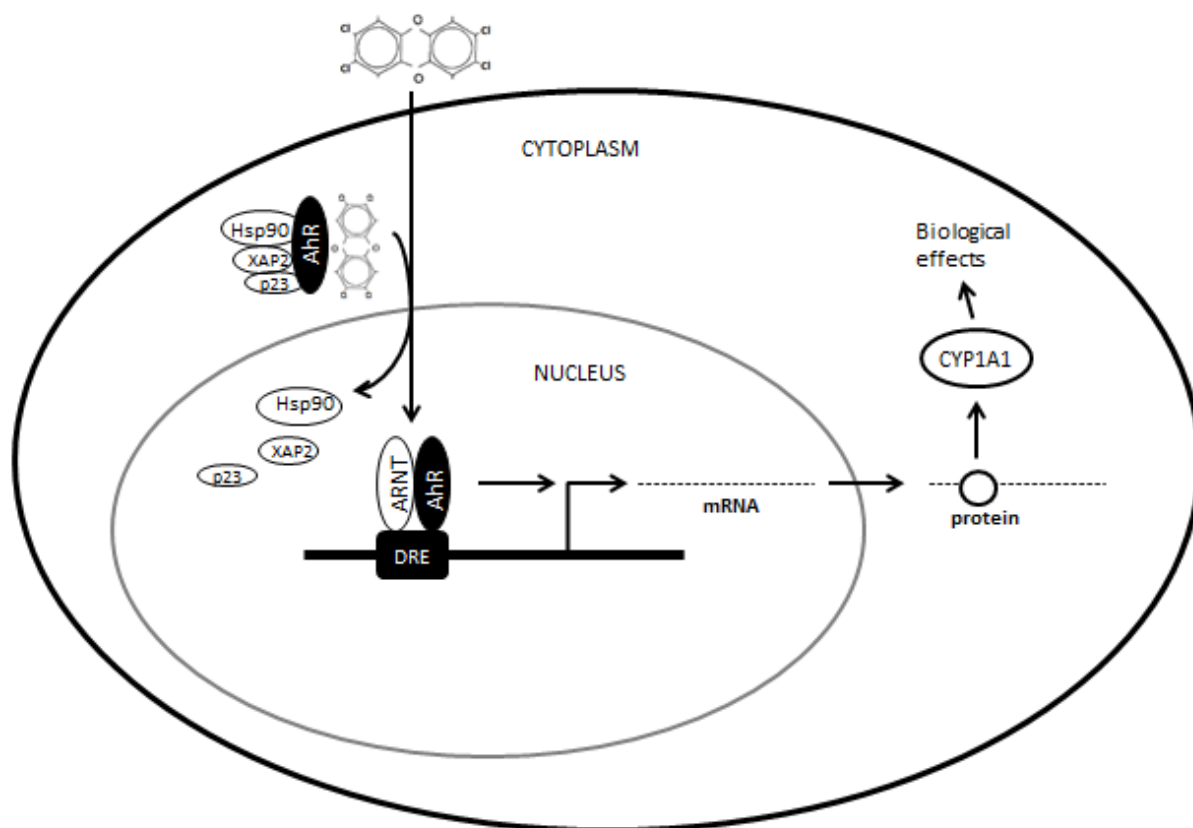


Figure 1.3 Mode of action of PCDD/Fs in the cell. Aryl hydrocarbon receptor (AhR) binding by PCDD/F mediates transcription of xenobiotic metabolising enzymes. Adapted from Zhou *et al.* (2010).

The CYP genes encode enzymes for the cytochrome P-450 system, an important component of microsomal mixed-function oxidation (MFO) reactions responsible for phase I biotransformation of lipophilic xenobiotics such as PCDD/Fs (Andersson and Förlin 1992, Nebert and Gonzalez 1987, Oinonen and Lindros 1998). Microsomes are found embedded in the smooth endoplasmic reticulum of a variety of tissues, with a particularly large abundance in the liver of vertebrates (Andersson and Förlin 1992, Oinonen and Lindros 1998). Expression of cytochrome (CYP) P450 enzymes occurs following transcription. CYP1A1 enzymes play a role in phase I metabolism which acts to convert toxicants into a polar form, allowing phase II

enzymes to produce a water-soluble conjugation product that is non-toxic and readily excreted (Andersson and Förlin 1992, Hodgson and Dauterman 1980, Whitlock *et al.* 1996).

1.3.3 Toxic Effects of PCDD/Fs

It has been well demonstrated that initiation of the AhR transcription factor by PCDD/Fs can lead to altered gene expression and toxicity, which can be broadly grouped into the following categories: reproductive toxicity, neurotoxicity, immunotoxicity, tumor promotion, and genotoxicity (Hahn 2001, Mandal 2005). Effects can occur in multiple organs, with the level of response dependent on the type of cell, tissue, age, sex, species and duration of exposure (Mandal 2005). Structural and functional differences in toxic response mechanisms, from receptor binding to transcription, have been demonstrated between mammalian and fish AhR as well as between different species (Hahn 2001, Wiseman 2007). Dose-response studies suggest fish are among the most sensitive organisms to PCDD/F exposure with a wide range of responses observed among species (Hahn 2001).

1.3.4 Toxic Equivalency (TEQ)

PCDD/Fs are present in the environment in complex mixtures, containing multiple congeners with different molecular structure and size, resulting in differences in toxicity. A toxic equivalency factor (TEF) is used to relate the toxicity of individual congeners to the toxicity of the most toxic congener, 2,3,7,8-TCDD. The TEF measures receptor binding to identify a ratio of toxicity between the congener of interest and 2,3,7,8-TCDD, which is set at 1 (Clemons *et al.* 1997, Fletcher and McKay 1993). The contribution of each compound to the toxicity of the mixture can subsequently be determined and the toxic equivalents (TEQ) for each compound can be added to determine the overall toxicity of the mixture using the following equation where the

concentration (C) of a particular congener (denoted as n) is multiplied by its TEF (Clemons *et al.* 1997, Fletcher and McKay 1993):

$$TEQ = \sum C_n \times TEF_n$$

TEQ values are commonly calculated using 2005 TEF_{mammal} and 1998 TEF_{fish} values (Table 1.1) reported by the World Health Organization (WHO) (Van den Berg *et al.* 1998, Van den Berg *et al.* 2006).

Table 1.1 Toxic equivalency factors (TEF) of PCDD/F congeners for mammals and fish reported by the World Health Organization (WHO).

		Toxic Equivalency Factor (TEF)	
	Congener	Mammal ^a	Fish ^b
Dioxins	2,3,7, 8–TCDD	1	1
	1,2,3,7,8–PeCDD	1	1
	1,2,3,4,7,8–HxCDD	0.1	0.5
	1,2,3,6,7,8–HxCDD	0.1	.01
	1,2,3,4,6,7,8–HpCDD	0.01	0.001
	OCDD	0.0003	<0.0001
Furans	2,3,7,8–TCDF	0.1	0.05
	1,2,3,7,8–PeCDF	0.03	0.05
	2,3,4,7,8–PeCDF	0.3	0.5
	1,2,3,4,7,8–HxCDF	0.1	0.1
	1,2,3,6,7,8–HxCDF	0.1	0.1
	1,2,3,4,6,7,8– HpCDF	0.01	0.01
	OCDF	0.0003	<0.0001

^aVan den Berg *et al.* (2006)

^bVan den Berg *et al.* (1998)

1.4 Study sites

1.4.1 Jackfish Bay

Jackfish Bay (JFB) (48°50'N; 86°58'W) is located on the north shore of Lake Superior (Fig. 1.4). Since the mill opened in 1948 it has discharged untreated (1948–1978), primary

treated (1978–1989) or secondary treated (1989–present) effluent into Jackfish Bay (Table 1.2). Effluent from AV Terrace Bay (formerly Terrace Bay Pulp Inc.) is routed through a canal into Blackbird Creek where it eventually discharges into Moberly Bay, the west arm of Jackfish Bay (Fig. 1.4). The effluent plume historically remained in contact with both shorelines for approximately 300 m, after which effluent flowed primarily along the west shore where it extended up to 5 km at 1% dilution (Comba *et al.* 1994, Sibley *et al.* 2001). The pulp mill is the only current source of contamination to Jackfish Bay; it does not receive any additional industrial or municipal effluent (Jackfish Bay RAP Team, 1991). Along the east shore of Jackfish Bay is the historic town of Jackfish which was built to serve the railway running along the shoreline of the bay. The town was abandoned following a major fire in the 1960s and was never rebuilt. There is also a small residential landfill located near the ghost town. Neither the landfill or the railway is considered to pose an ongoing threat of contamination to Jackfish Bay.

As the area of Lake Superior surrounding Moberly Bay is >12 m in depth, white sucker collected from Moberly Bay are considered to be residents of the bay (BEAK 1995, McMaster *et al.* 1991). Due to the lack of spawning habitat, white sucker residing in Moberly Bay migrate through Tunnel Bay to Sawmill Creek, a small stream connected to Jackfish Lake, during the spring (Fig. 1.4). Neither Jackfish Lake nor Sawmill Creek receive industrial or municipal effluent contamination.

Since 2005 the mill has experienced a number of closures and production changes, including permanent closure of its hardwood line (no. 1 mill), a 30% decrease in production and decreased effluent discharge (Table 1.2). In the fall of 2011 the mill was discharging effluent into the bay. Production ceased from October 2011 until October 2012, after which time the mill

reopened under new ownership. Future plans for the mill include conversion to a dissolving pulp process over the next three years (Aditya Birla 2012).

Mountain Bay (MTB) (46°56'N; 87°58'W), located approximately 100 km west of Jackfish Bay in Lake Superior, has historically been used as a reference site for fish studies conducted at Jackfish Bay (BEAK 1996, McMaster *et al.* 1991, Munkittrick *et al.* 1992a, Munkittrick *et al.* 1991, Servos *et al.* 1994). Mountain Bay serves as a good reference site because it receives no industrial or municipal effluent contamination and offers habitat similar to Jackfish Bay. During the spring, white sucker from Mountain Bay migrate through nearby Little Gravel River to reach suitable spawning habitat.

Santoy Bay (48°46'N; 86°54'W) located immediately adjacent to Jackfish Bay (Fig. 1.4) has been the primary reference site used for benthic invertebrate community studies, including use in the EEM program. Sediment in Santoy Bay has historically been characterized as sandy in the near shore reaches with coarse sand and gravel leading to mud in the deeper areas within the bay (BEAK 2000, Sherman *et al.* 1990, Jackfish Bay RAP Team, 1991). The Steel River, a salmonid spawning river, flows into the center of the bay. Santoy Bay receives no industrial or municipal effluent contamination. Tunnel Bay (part of Jackfish Bay) has been used as an additional reference site for the invertebrate community assessment component of EEM studies. Differences in community structure between the two reference sites were minimal and not considered to be ecologically meaningful, when the finer substrate and greater total organic carbon (TOC) content of sediments in Tunnel Bay were considered compared to Santoy Bay (BEAK 2000).

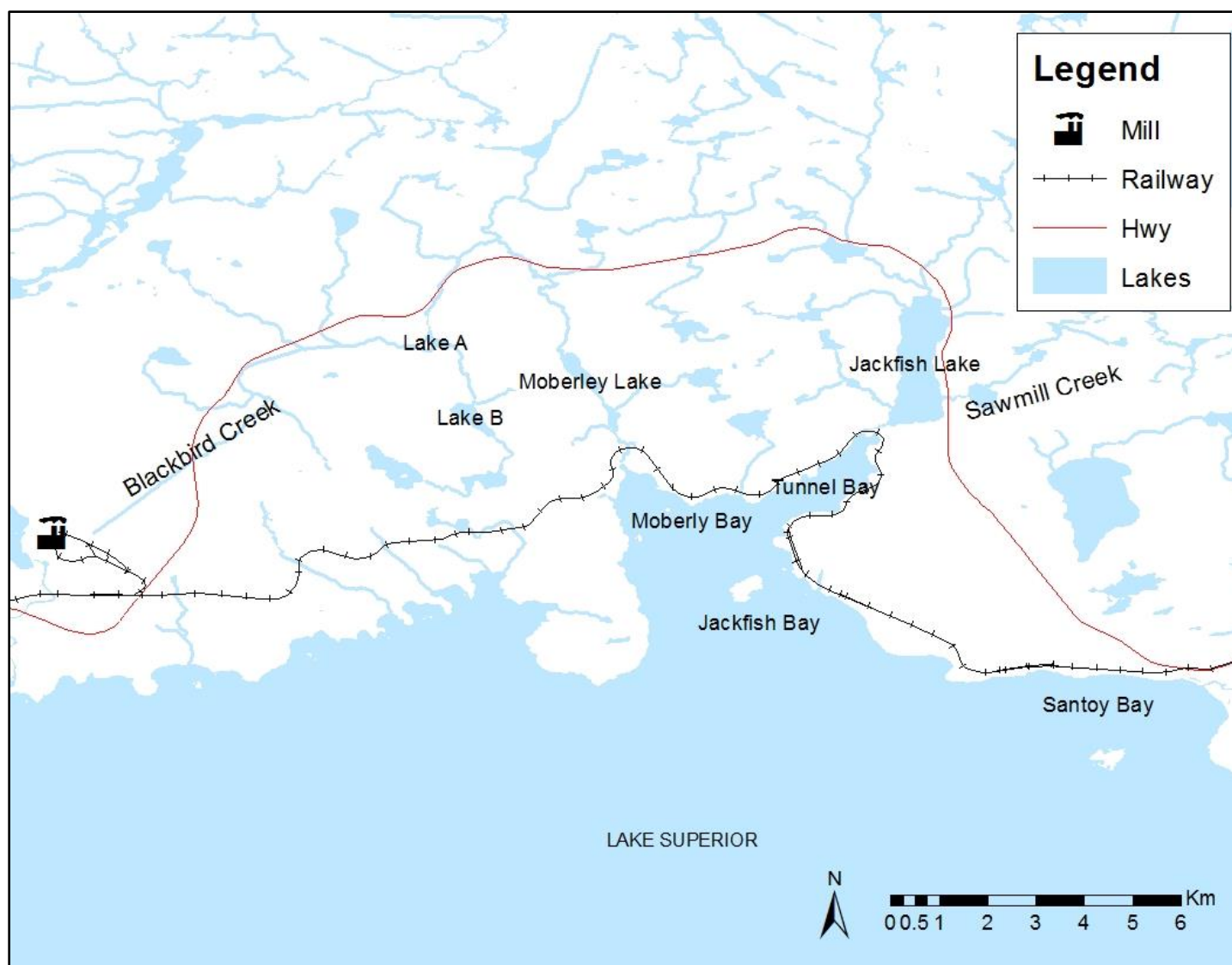


Figure 1.4 Map of study site at Jackfish Bay and sediment reference site, Santoy Bay, on Lake Superior. A second reference site, Mountain Bay, is located approximately 60 km west on Lake Superior.

Table 1.2 Summary of process changes at the bleached kraft pulp mill in Terrace Bay, ON including changes to the hardwood (no.1 mill) line, softwood (no. 2 mill) line and bleaching sequence (Adapted from Bowron *et al.* (2009)).

Year	Process Change
1948	Mill began operations as an unbleached kraft mill with capacity of 270 ADMT/d (hardwood only). No effluent treatment.
1958	Cl added to bleaching circuit.
1959	Cold bleaching introduced.
1972	Mill expansion to fully bleached two-line 'hot' kraft mill with increased capacity of 400 ADMT/d. From 1972–1978 the mill used a mercury anode to produce chlorine gas for the bleaching process.
1973	New recovery boiler installed.
1975	Blackbird Creek diverted to bypass Lake A. Production capacity of 435 ADMT/d
1978	New bleaching and finishing plant brought online, increasing total capacity to 1135 ADMT/d; primary treatment facility installed incorporating two reactor clarifiers; no.2 mill and dry debarking added.
1979	Clarifier installed for alkaline sewer.
1981	Major reconstruction after a fire, including installation of: condensate stripper, turpentine decanter, NCG collection and destruction system, bypass domestic sewage treatment plant and clarifier screening system.
1982	Installed cooling water recycle system for kiln/causticizing area.
1984	Spill control system completed in no.2 mill; improved soap recovery; increased ClO ₂ substitution; no.1 mill dedicated to hardwood; polymer feed system added to alkaline clarifier; additional clarifier added and improvements to no.2 brownstock washing.
1985	No.2 brownstock closure; spill control system completed in no.1 mill; extraction oxidation (E ₀) stage added to no.2 bleachery; new instrumentation for bleachery to decrease chemical use
1986	Completed modification of no.1 brownstock washers: improved soap recovery, foam control and vacuum improvements.
1989	Secondary treatment installed: aerated stabilization basin (ASB); papricycle stage (wash cycle) added.
1990	Increased ClO ₂ substitution to approximately 50%; hypochlorite replaced with papricycle; new control system.
1991	Chlorine strength analyzers and recirculation piping installed; new chip thickness screening plant and hot water stave replaced; increased softwood production by 45 ADMT/d.
1993	Concentrator for no.2 mill recovery boiler (black liquor): steam operated, two effect concentrator increased liquor solids from 63% to 75%; and eliminated cascade evaporator; low liquor concentration and moist, low temperature combustion air from the cascade evaporators leads to the formation of TRS compounds resulting in improved air quality; increased ClO ₂ substitution from 50% to 70%.
1994	Replaced 250 m section of creosote treated wooden stave piping.

Table 1.2 cont'd Summary of process changes at the bleached kraft pulp mill in Terrace Bay, ON including changes to the hardwood (no.1 mill) line, softwood (no. 2 mill) line and bleaching sequence (Adapted from Bowron *et al.* (2009)).

Year	Process Change
1995	Updated ClO ₂ generator from the R3 process to R8 allowing the mill to continually produce elemental chlorine free (ECF) pulp in both no.1 and no.2 mills and lowering the discharge of chlorinated organics; diversion pond created to collect discharged untreated process water in the event of a spill and serve to collect storm water; no untreated effluent has bypassed the ASB and no reported spills.
1996	More mature wood (purchased), less lignin, hence less sulfur lignin by-products.
1997	Hydrogen peroxide use started in No. 2 mill in bleaching sequence E ₂ stages. Average monthly production 1260 ADMT/d.
1998	Hydrogen peroxide use started in no. 2 mill in bleaching sequence E ₀ stage; no.1 mill switched production to batch softwood for periodic short campaigns and to 100% ECF bleaching in October 1998.
1999	No.2 mill switched over to 100% ECF bleaching in April 1999.
2000	New brownstock washing showers in September; mill producing bleached sulphate pulp at an average rate of 1260 ADMT/d.
2003	Acid activated oxygen bleaching stage with two reactors installed in third stage of no.2 bleach plant process; modification made to third stage of bleaching process reduced adsorbable organic halides (AOX) by 47%.
2005	No.1 mill shutdown April 1, 2005.
2006	No.2 mill shutdown February 2006. Buchanan Forest Products Ltd. announced takeover of the Terrace Bay pulp mill and woodland operations. Formal transfer between Neenah and Buchanan was finalized in fall 2006 and mill re-opened (softwood only) as Terrace Bay Pulp Inc. on September 20, 2006.
2007	OMOE issued an order to Terrace Bay Pulp Inc. requiring them to submit either an application to approve their bark resources pile (BRP) as a waste transfer/processing site (including a schedule to remove the material from the site within 5 years), or alternatively provide supplemental information to allow for the designation/closure of the area as a waste disposal site, by July 31, 2007. The order also required the company to install a new groundwater monitoring well and conduct groundwater and surface water monitoring in the vicinity of the BRP, with a monitoring report to the ministry by March 2008.
2008	New steam turbine in operation; one month "downtime" in November to December 2008.
2009	Mill reduced operations in no. 2 mill and shutdown no. 1 mill indefinitely in January, followed by complete mill closure in February 2009; company filed for creditor protection March 2009.
2010	Mill reopened October 2010.
2011	Mill announced temporary shutdown in October 2011.
2012	Mill purchased by Aditya Birla Group in July 2012. The mill will be operated under the Canadian subsidiary AV Terrace Bay. Operation of the NBSK process commenced in October 2012 with plans to convert the facility to a dissolving pulp mill over a 2–3 year period.

(Aditya Birla 2012, BEAK 1998, Bowron et al., 2009, Comba et al. 1994, Servos et al. 1997, Sibley et al. 1998, Jackfish Bay RAP Team, 1991, 1998)

1.4.2 Smooth Rock Falls

The Moose River basin located in northeastern Ontario drains into James Bay (Fig. 1.5). The Mattagami River is one of the major tributaries of the system, with a drainage area of 41,672 km² and includes hydroelectric facilities, water storage reservoirs and a bleached kraft pulp mill in the town of Smooth Rock Falls (Munkittrick *et al.* 2000). The dam and generating station at Smooth Rock Falls historically provided electrical power to the mill. The pulp mill at Smooth Rock Falls (49°17'N; 81°37'W) was operational from 1918 until 2006 but was not converted to a bleached kraft process until 1927 (Table 1.3). During mill operation, effluent was discharged approximately 0.15 km downstream of the dam and 31.7 m from the eastern shoreline of the Mattagami River (ACRES 1996, Munkittrick *et al.* 2000). It is suspected that effluent from the mill remained at > 1% dilution for 64 km downstream until it reached the confluence with the Kapuskasing River which contains a thermo-mechanical pulp mill at the town of Kapuskasing (ACRES 1996, Stantec 2005). A small sewage treatment plant serving the town of Smooth Rock Falls discharges into a tributary of the Mattagami River which joins the main stem approximately 200 m downstream of the mill effluent discharge pipe (ACRES 1996). Upstream of the dam at Smooth Rock Falls the reservoir forms a deep depositional area with substrate dominated by organic matter, silt and clay. The river downstream of the mill is shallow with sandy substrate and no depositional areas. Below Cypress Falls (30 km downstream) the substrate becomes dominated by clay.

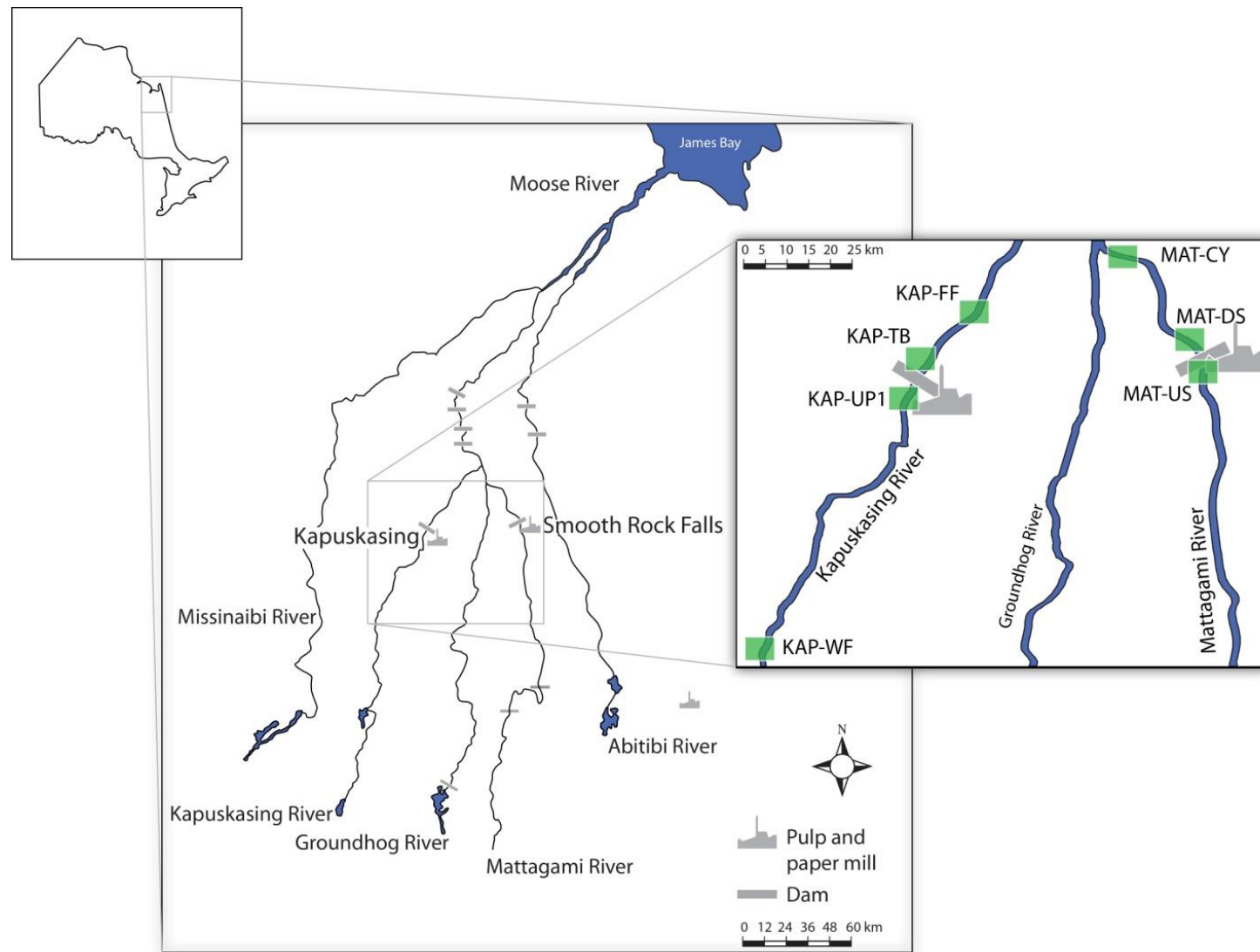


Figure 1.5 Map of Moose River basin with enlargement of the Kapuskasing, Groundhog and Mattagami Rivers, including pulp and paper mills and hydroelectric dams (Arciszewski *et al.* submitted)

Table 1.3 Summary of process changes at the bleached kraft pulp mill in Smooth Rock Falls, ON.

Year	Process Changes
1917	Construction complete on the Mattagami Pulp and Paper Company mill (unbleached kraft process) and dam.
1927	Process converted to bleached-kraft.
1976	Primary treatment installation (mechanical clarifier).
1986	Log drives on Mattagami river ceased in 1986.
1991	ClO ₂ generators installed.
EEM Cycle 1 (1992-1996): Primary treatment only	
	Mill and dam owned and operated by Malette Kraft Pulp and Power.
1992	ClO ₂ substitution increased to 100% (up from 16 %); bleaching sequence changed from CdEopDED to DEopDEpD.
1993	O ₂ delignification installed; average monthly production 344 ADMT/d
1994	Secondary treatment (aeration stabilization basin with 7.5 d retention time) installed and operational by October.
1995	Secondary treatment operating efficiently by summer.
EEM Cycle 2 (1996-2000) - Primary and secondary treatment	
	Pressure diffuser washer added to pulping line.
	Reduced chemical oxygen demand (COD) & biological oxygen demand (BOD) of influent.
	BOD reduction program: contaminated condensate re-use; changes in liquor washing protocols; recirculating Byrd filter rejects
EEM Cycle 3 (2000-2004): Primary and upgraded secondary treatment	
	Mill and dam owned and operated by Tembec Smooth Rock Falls Pulp and Paper Mill.
1998	Average monthly production 554 ADMT/d.
1999	Water use reduction (goal: 43 000 m ³ /d); enhanced spill detection; landfill leachate diverted to aerated stabilization basin (ASB); influent monitoring shows no significant increase in formaldehyde residuals.
2000	Expanded secondary treatment system from 2-cell to 4-cell ASB; average monthly production 552 ADMT/d.
1999-2001	Periodic (1-4 times/yr) treated sewage sludge added to ASB - practice halted due to nutrient loading.
2001-2002	Adding formaldehyde to ClO ₂ to increase bleaching efficiency (trials)
EEM Cycle 4 (2004-2007)	
2004	Liquid nutrient addition system operational in November to provide a consistent feed rate and avoid fluctuations; sewage discharged immediately downstream of outflow but loading is less than 1% of phosphorus
2006	Production halted in July 2006

(ACRES 1996, ESG 2000, Stantec 2004b, 2007)

1.5 Overview of evidence for environmental impact of bleached kraft mill effluent in two northern Ontario mills

To address concerns surrounding the effects of effluent discharged from Canadian pulp mills on aquatic ecosystems, the relationship between environmental responses and pulping processes at 10 mills across Ontario was examined (Munkittrick *et al.* 1994, Robinson *et al.* 1994, Servos *et al.* 1994, van den Heuvel *et al.* 1994). The most dramatic physiological responses were observed in fish collected downstream of the Smooth Rock Falls and Terrace Bay, ON bleached kraft pulp mills, which at the time of the study employed primary and secondary treatment, respectively (Munkittrick *et al.* 1994).

1.5.1 Fish

A variety of biological responses were reported in fish collected from the receiving environments of the bleached kraft pulp mills at Smooth Rock Falls and Terrace Bay. Observed responses included: decreased gonad size, delayed sexual maturity, depressed secondary sexual characteristics in males, a reduction in circulating sex steroids, increased liver size, and induction of MFO activity (BEAK 1996, McMaster *et al.* 1991, Munkittrick *et al.* 1991, Munkittrick *et al.* 1994, Nickle *et al.* 1997). MFO induction has historically been used as a biomarker for PCDD/F contamination. At Smooth Rock Falls, MFO induction has been reported in white sucker > 30 km downstream of the pulp mill (Munkittrick *et al.* 2000). However, further investigations measuring MFO induction in fish exposed to a variety of mill effluents, with and without chlorine bleaching, determined PCDD/Fs were associated with only a portion of MFO induction (Lindström-Seppä *et al.* 1992, Nickle *et al.* 1997, Servos *et al.* 1994, van den Heuvel *et al.*

1994). Many of the chemicals responsible for MFO induction observed in exposed fish remain unidentified (Hewitt *et al.* 2006, Kovacs *et al.* 2011).

Analysis of PCDD/F concentrations measured from BKME in the late 1980s and early 1990s demonstrated a unique congener “bleaching pattern” (Sherman *et al.* 1990, Swanson *et al.* 1988). A comparison of PCDD/F congener patterns from mill effluent, as well as caged and wild biota collected from Jackfish Bay, suggested active uptake of pulp mill-derived PCDD/Fs (Sherman *et al.* 1990). Mean TEQs for male white sucker liver tissue, calculated using international mammalian TEF (I-TEF) values (Kutz *et al.* 1990), were reported as high as $124 \pm 96.7 \text{ pg}\cdot\text{g}^{-1}$ and $86.3 \pm 23.4 \text{ pg}\cdot\text{g}^{-1}$ downstream of the Smooth Rock Falls and Terrace Bay mills, respectively. This compares to an average TEQ of $2.09 \pm 1.80 \text{ pg}\cdot\text{g}^{-1}$ at three reference sites (Servos *et al.* 1994). Lower TEQ values were detected in fish collected in the receiving environment of bleached kraft mills employing higher ClO_2 substitution as well as secondary treatment (Servos *et al.* 1994). The addition of secondary treatment allows PCDD/Fs to associate with organic matter that will settle in retention ponds. Despite installation of secondary treatment at the Terrace Bay pulp mill in 1989, no immediate decline in PCDD/F concentrations was observed, compared to previous years (Servos *et al.* 1994).

1.5.2 Sediment

The benthic environment in Jackfish Bay was identified as severely impacted during multiple surveys conducted between 1969 and 1987 (Jackfish Bay RAP Team, 1991). Sibley *et al.* (2000) further classified three distinct regions within Jackfish Bay: highly degraded sediment, 300–1200 m from Blackbird Creek; an adjacent nutrient enriched area; and the remaining areas of Jackfish Bay, including Tunnel Bay. Unique invertebrate communities have been

characterized in each region, with benthic community structure significantly related to organic matter and extractable organic chlorine (EOCl) concentration (Sibley *et al.* 1997, 1998, 2000). Within the highly degraded zone, high sediment toxicity and severely altered benthic community structure have been identified between 300–400 m with low to moderate impacts observed up to 1200 m from Blackbird Creek (Sherman *et al.* 1990, Sibley *et al.* 1997, 1998, 2000, Stewart and Rashid 2011, Jackfish Bay RAP Team, 1991). Similarly, at Smooth Rock Falls severe impairment of aquatic invertebrate populations was observed for at least 16 km downstream of the mill (Brousseau and Goodchild 1989).

Recent improvements have been observed in the benthic community within Jackfish Bay (Owens *et al.* 1994). However, PCDD/F concentrations in sediment collected from Moberly Bay remain elevated above Provincial Sediment Quality Guidelines (PSQG) and continue to be listed as impaired (Milani 2009).

1.6 Objectives

The overall objective of this thesis was to examine the temporal changes in PCDD/F contamination of white sucker and sediment historically exposed to BKME following more than a decade of process changes and mill closures at two locations in northern Ontario. The specific objectives of the study were:

- 1) Determine whether TEQs remain elevated compared to background (reference) levels in male white sucker liver tissue collected in 2011 and 2012 from Jackfish Bay, Lake Superior and the Mattagami River, downstream of the mill at Smooth Rock Falls.
- 2) Examine whether current PCDD/F concentrations and individual congener levels measured in male white sucker liver reflect historical mill operations and sediment contamination

profiles from the receiving environment of the bleached kraft pulp mills in Terrace Bay and Smooth Rock Falls, Ontario.

Chapter 2 Historical trends of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in white sucker (*Catostomus commersoni*) liver and dated sediment cores from Jackfish Bay, Lake Superior

The contributing authors of this chapter are:

Cater, Shari C.¹, Roland I. Hall¹, Kelly R. Munkittrick², Mark E. McMaster³, and Mark R. Servos¹

1 Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1

2 Department of Biology, University of New Brunswick, Saint John, NB E2L 4L5

3 Ecosystem Health Assessment, Environment Canada, Burlington, ON L7R 4A6

- Shari Cater: M.Sc. candidate who researched, collected, analyzed and wrote the paper
- Roland Hall: Assisted with development of core chronology
- Kelly Munkittrick: Assisted with field work, past and present, and provided general advice
- Mark McMaster: Committee member of Shari Cater. Assisted with field work, past and present, and provided general advice
- Mark Servos: Supervisor of Shari Cater. Collected historical data, assisted with field work, ideas, research direction, editing, and provided general advice.

2.1 Summary

Despite new effluent regulations and an industry shift toward total chlorine free (TCF) bleaching, previously released polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) produced by pulp mills, during the period from the 1960s to early 1990s, may remain a concern for aquatic ecosystems due to their tendency to persist in sediment and bioaccumulate in aquatic biota. PCDD/Fs were measured in fish and sediment collected in the receiving environment of the bleached kraft pulp mill in Terrace Bay, Ontario to document the spatial and temporal pattern of contamination in relation to historical changes in mill processes and operations. The mill discharges effluent into Jackfish Bay, on the north shore of Lake Superior, where the effects of mill effluent have been studied since the late 1980s. PCDD/Fs measured in male white sucker (*Catostomus commersoni*) captured from Jackfish Bay reached peak toxic equivalents (TEQ) of $97.8 \pm 28.2 \text{ pg} \cdot \text{g}^{-1}$ in spring 1991. A decreasing trend has been observed in recent years with fish collected in fall 2011 ($5.82 \pm 3.42 \text{ pg TEQ} \cdot \text{g}^{-1}$) and 2012 ($3.34 \pm 2.05 \text{ pg TEQ} \cdot \text{g}^{-1}$) approaching PCDD/F contamination levels observed in reference fish ($1.80 \pm 0.16 \text{ pg TEQ} \cdot \text{g}^{-1}$) collected from Mountain Bay, Lake Superior during the same time period. Although below consumption guidelines, trace levels of PCDD/Fs persist in fish collected from Jackfish Bay. It was suspected that these concentrations would reflect sediment contamination from PCDD/Fs deposited during historical mill operations. The PCDD/F congener profile observed in white sucker was consistent with sediment samples collected from the depositional area of Moberly Bay (part of Jackfish Bay), where 2,3,7,8-TCDD and 2,3,7,8-TCDF dominated the PCDD/F contaminant profile. Results from fish and sediment demonstrated the presence of a unique PCDD/F bleaching pattern in the receiving environment of Jackfish Bay. The congener profile transitioned to a pattern more indicative of air emissions with increased distance from the

pulp mill. PCDD/F contamination of a dated sediment core collected from Moberly Bay illustrated a similar pattern of decreasing TEQ over time which could be attributed to mill process changes. Surface sediments collected throughout Jackfish Bay demonstrated elevated TEQ compared to two reference locations on Lake Superior. Within the depositional area of Moberly Bay TEQ values measured in surface sediment were above the Canadian Council of Ministers of the Environment (CCME) probable effects level (PEL) of 21.5 pg TEQ·g⁻¹. This guideline was developed to evaluate potential for PCDD/F exposure to cause adverse biological effects. Temporal patterns of PCDD/F contamination observed in white sucker and sediment suggest that particulate organic matter (POM) and dissolved organic matter (DOM) historically provided the dominant source of PCDD/Fs to fish, with a small contribution from surface sediment. PCDD/F concentrations in sediment from Jackfish Bay, especially in Moberly Bay, remain elevated from historical contamination; however, these are not highly bioavailable and PCDD/F concentrations in white sucker are currently only slightly elevated compared to reference fish from Lake Superior.

2.2 Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are persistent organic pollutants formed as by-products of pulp and paper production. They have received international attention due to their high toxicity and long half-life in fish and sediment (Hahn 2001, Jones *et al.* 2001, Parrott *et al.* 1995, Segstro *et al.* 1995). The presence of PCDD/Fs has been linked to the bleaching process in pulp and paper production, particularly the historical use of molecular chlorine (Cl₂) as a bleaching agent (Amendola *et al.* 1989, Axegård and Renberg 1989, Berry *et al.* 1989, Swanson *et al.* 1988). The implementation of new effluent regulations

under the Canadian Environmental Protection Act (CEPA) and development of the Environmental Effects Monitoring (EEM) program in 1992 contributed to an industry shift toward the use of elemental chlorine free (ECF) and total chlorine free (TCF) bleaching practices (Luthe 1998, McDonough 1995). The result of these process changes was a rapid decline of PCDD/Fs in effluent from mills across Canada (Luthe *et al.* 1992, Servos *et al.* 1997, Yunker *et al.* 2002). The persistence of highly chlorinated PCDD/F congeners may allow them to remain in sediment for long periods of time, providing a potential source of contamination to the receiving environment (Segstro *et al.* 1995). As they are highly lipophilic and have a tendency to bioaccumulate in aquatic biota, PCDD/Fs pose a concern to the health of aquatic ecosystems and potential restrictions on human consumption of resident fish species (Muir and Servos 1996).

Jackfish Bay, Lake Superior was listed as a Great Lakes Area of Concern (AOC) in 1987 due to the effluent discharged from the bleached kraft pulp mill located in Terrace Bay, ON. The AOC included Jackfish, Moberly and Tunnel Bays, as well as 14 km of Blackbird Creek which receives effluent from the mill (Environment Canada and OMOE 2011). The benthic environment in Jackfish Bay, especially Moberly Bay, has been identified as severely impacted with impaired benthic invertebrate community structure (Sibley *et al.* 1997, Sibley *et al.* 2000, Stewart and Rashid 2011, Jackfish Bay RAP Team, 1991). Studies conducted in the late 1980s and early 1990s identified a number of responses in fish collected from Jackfish Bay. These included: delayed sexual maturity; depressed secondary sex characteristics in males; reduced circulating steroid hormone levels; metabolic disruption, characterized by increased condition, increased liver size and decreased gonad size; and induction of liver detoxification enzymes (McMaster *et al.* 1991, Munkittrick *et al.* 1991, Munkittrick *et al.* 1994). At the same time elevated PCDD/F concentrations were measured in *Mysis relicta* and white sucker (*Catostomus*

commersoni) collected from Jackfish Bay, with PCDD/F congener patterns consistent with the distinct bleaching pattern found in mill effluent (Servos *et al.* 1994, Sherman *et al.* 1990). A core profile collected from Moberly Bay by Sherman *et al.* (1990) demonstrated an abrupt appearance of tetrachlorodibenzofuran (TCDF) around 1973, which remained elevated at the time of sampling in 1988. The sudden formation of TCDF corresponds to process upgrades at the mill, involving the use of oil-based defoamers in brown stock washers which contained furan precursors (Allen *et al.* 1989, Sherman *et al.* 1990). Increases in temperature use in the chlorine bleaching process likely contributed to favorable conditions for the formation of highly chlorinated PCDF congeners (Smook 1992, Jackfish Bay RAP Team, 1991).

Long-term studies have continued at Jackfish Bay over the last 25 years as the mill has undergone a number of process changes and closures (Bowron *et al.* 2009, McMaster *et al.* 2006). Upgrades to mill processes included installation of secondary treatment and removal of PCDD/Fs through increased chlorine dioxide (ClO₂) substitution. These have resulted in reduced effluent toxicity, improved liver and gonad size and partial recovery of reproductive function (Munkittrick *et al.* 1997). However, complete recovery of fish populations during mill operation has not been observed (Bowron *et al.* 2009) and the responsible chemical(s) remain unidentified (Hewitt *et al.* 2006, Kovacs *et al.* 2011). In early 2005 the mill went through the first of many temporary closures. The hardwood line shutdown in April 2005, followed in February 2006 by the softwood line. By fall 2006, mill ownership had changed and the softwood line was reopened. In early 2009 the mill went through another period of reduced operations, followed by a complete mill closure, reopening in October 2010. As of 2010, Jackfish Bay has been recognized as an Area in Recovery (Environment Canada and OMOE 2011). Despite the observed signs of ecosystem recovery, the benthic and fish community structures remain

impaired throughout Jackfish Bay (Milani 2009). Since reopening in 2010, the mill has undergone a 12 month shutdown from October 2011 to October 2012 along with a change in ownership. The facility is expected to be converted to a dissolving pulp process over the next 2–3 years (Aditya Birla 2012).

The objective of this study was to re-examine how PCDD/F concentrations have changed in fish and sediment in response to mill process changes and temporary closures. The concentrations of PCDD/Fs in white sucker collected from Jackfish Bay in 2011 and 2012 are compared to historical data collected in the early 1990s. The PCDD/F congener profiles of fish are also compared to PCDD/F contamination of dated sediment cores and surface sediments collected throughout Jackfish Bay in 2012.

2.3 Materials and Methods

2.3.1 Study site

The bleached kraft pulp mill in Terrace Bay discharges effluent into Blackbird Creek where it travels 14 km to reach Moberly Bay, the west arm of Jackfish Bay on Lake Superior. The pulp mill is the only current source of contamination to Jackfish Bay; it does not receive any additional industrial or municipal effluent (Jackfish Bay RAP Team, 1991). Since the mill began operations in 1948 it has experienced a number of process changes, including multiple periods between 2005 and 2012 during which time the facility has been shut down (Table 1.2, Chapter 1). Production ceased from October 2011 until October 2012 when the mill reopened under new ownership (Aditya Birla 2012).

White sucker residing in Moberly Bay migrate during the spring through Tunnel Bay (part of Jackfish Bay) to Sawmill Creek, a small stream connected to Jackfish Lake. Tunnel Bay has historically received < 1% effluent pollution and has been used as an additional reference site for invertebrate community assessment in EEM (BEAK 1998, 2000). Neither Jackfish Lake nor Sawmill Creek receive industrial or municipal effluent contamination.

Mountain Bay (MTB), located approximately 60 km west of Jackfish Bay has historically been used as a reference site for fish studies conducted at Jackfish Bay (BEAK 1996, McMaster *et al.* 1991, Munkittrick *et al.* 1991, Servos *et al.* 1994). Santoy Bay, located immediately adjacent to Jackfish Bay, in Lake Superior, was used in this study as a reference site for PCDD/F analysis of sediment (as was done by Sherman *et al.*, 1990). Neither Mountain Bay nor Santoy Bay receives industrial or municipal effluent contamination.

2.3.2 Fish collection and sampling procedures

Fish collections occurred at Jackfish Bay and Mountain Bay during the following time periods: August 28–31, 2011; April 25–30 2012; August 22–24, 2012. White sucker collected during the gonadal recrudescence period (fall) were captured using gill nets (3.5 and 4 inch mesh size). Pre-spawning white sucker were collected in the spring from spawning streams: Sawmill Creek (Jackfish Bay) and Little Gravel River (Mountain Bay), using overnight hoop nets. For each site, 20 males and 20 females were sampled as part of a long term monitoring program. Each fish was rendered unconscious by a sharp blow to the head, followed by determination of sex, fork length (± 0.1 cm), body weight (± 0.1 g), gonad weight (± 0.01 g) and liver weight (± 0.01 g). The left operculum was removed and stored at -20°C for age determination. A sample of liver tissue ($\sim 5\text{--}10$ g) was removed from each fish, wrapped in foil and frozen.

at -20°C. Liver samples from male white sucker were randomly selected from Jackfish Bay (n = 6) and Mountain Bay (n = 3) during each sampling period for PCDD/F analysis.

2.3.3 Sediment Collection

Sediment sampling locations were selected to represent the three depositional basins in Jackfish Bay (Tunnel Bay, Moberly Bay and Jackfish Bay) and two reference sites outside the bay (Santoy Bay and Mountain Bay). In the near shore areas surface sediment samples were collected using an Ekman grab. Ensuring the sample was not mixed, a 5 cm core was obtained from the middle of each grab and sectioned at 1 cm intervals. In the depositional areas core samples were collected using a gravity corer with an internal diameter of 15.25 cm. Each core was sectioned into 1 cm intervals up to the depth at which a change in sediment composition to the original silt-clay sediment was visible (~20–30 cm). All sediment samples were stored in certified clean wide mouth amber glass jars and frozen (-20°C) immediately upon collection.

2.3.4 ²¹⁰Pb Dating of Sediment Cores

Total wet weight of each sediment sample (1 cm core section) was determined prior to subsampling. A 0.5 ± 0.05 g (wet wt.) mixed subsample of sediment from each 1 cm section was collected to determine water (post 90°C, 24 h), organic matter (post 550°C, 1 h) and carbonate (post 950°C, 1 h) content using a standard sequential loss on ignition (LOI) procedure (Dean, 1974; Jarvis et al., 2006). Measurements of wet weight and water content were used to calculate cumulative dry mass which was required to develop core chronology.

Each core was dated by gamma ray spectrometric determination of ²¹⁰Pb, ¹³⁷Cs and ²²⁶Ra (inferred from the mean of ²¹⁴Bi and ²¹⁴Pb) activity at continuous 1 cm intervals. Subsamples (3–

4 g dry wt.) of freeze-dried sediment were packed into pre-weighed tubes (SARSTEDT product No. 55.524) to a height of 35 mm. A TFA silicone septa (Supelco[®]) was placed over the sediment, followed by 1 cm³ epoxy resin. A three week equilibration period followed to allow ²²²Rn and its decay products to reach equilibrium with the parent isotope ²²⁶Ra. Samples were analyzed in an Ortec coaxial HPGe Digital Gamma Ray Spectrometer (Ortec GWL-120-15) interfaced with Maestro 32 software (version 5.32). Sample count times (1–5 d) were varied to allow net ²¹⁰Pb counts to reach levels > 10x the standard deviation of sample blanks to ensure precision (Currie 1968). Background counts were conducted at regular intervals (first and last samples of each core and every 6–8 samples).

The Constant Rate of Supply (CRS) model was used to develop the ²¹⁰Pb core chronologies as described by Appleby (2001). The CRS model requires an estimate of supported ²¹⁰Pb activity, as determined by the activity of ²¹⁴Bi and ²¹⁴Pb. It assumes a constant supply of ²¹⁰Pb while considering variations in the sedimentation rate (Appleby 2001). The mean value of all ²²⁶Ra determinations for a given core was considered representative of supported ²¹⁰Pb activity. Background depth (where total ²¹⁰Pb activity equals supported ²¹⁰Pb activity) was determined similar to that described by Binford (1990).

2.3.5 PCDD/F analysis

PCDD/F analysis was conducted by AXYS Analytical Services Ltd. (Sidney, BC) according to US EPA Method 1613B with some modifications (AXYS 2012, US EPA 1994). Briefly, liver (1–5 g wet wt.) or freeze dried sediment (4–10 g dry wt.) samples were homogenized and mixed in sodium sulfate, allowed to dry for 12–24 h and spiked with an internal standard mixture of ¹³C labelled isotopes. Samples were extracted for 18–24 h in a

Soxhlet extractor with 1:1 v/v dichloromethane: hexane (tissue) or 80:20 v/v toluene: acetone (sediment). Lipid removal and sample cleanup was conducted manually by gel permeation chromatography (GPC). The first column was a multi-layer acid-base silver nitrate (AgNO_3) silica column (20 g 44% tissue; 30 g 44% sediment), followed by a Florisil column (tissue) or copper column (sediment). Finally, activated alumina/carbon/Celite combination columns were used to isolate planar compounds. Extracts were concentrated to near dryness using nitrogen following cleanup. Lipid content of liver tissue was determined gravimetrically from the Soxhlet extracts. Sample analysis was performed with isotope dilution using a high resolution gas chromatography (HRGC)/ high resolution mass spectrometry (HRMS). Quantification of target analytes was determined according to USEPA Method 8290 and 8290A (US EPA 1994, 1998). Concentrations were corrected for the recovery of the ^{13}C labelled standards.

2.3.6 Statistical analysis

Data from male white sucker collected from Mountain Bay during spring 1993 were not included due to corruption of historical spreadsheets which prevented sample identification. In addition, one male fish was removed as an outlier from the analysis of white sucker collected at Jackfish Bay in fall 1993 because of unusually high detection limits.

Toxic equivalents (TEQ) were calculated using congener specific fish toxic equivalency factors (TEF) reported by the World Health Organization (WHO) (Van den Berg *et al.* 1998). TEQ values for tissue samples are reported as wet weight ($\text{pg}\cdot\text{g}^{-1}$ wet wt.) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ lipid) values, while sediment TEQs are reported on a dry weight ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and organic matter normalized ($\text{pg}\cdot\text{g}^{-1}$ OM) basis. Where PCDD/F concentrations were below detection limits (reported as ND), a value of one half of the sample detection limit (DL) was used

to calculate TEQ. Detection limits were not available for white sucker tissue samples collected during spring and fall 1995; TEQ values for these samples were calculated using 1993 mean detection limits (i.e., $ND_{1995} = \frac{1}{2} DL_{1993}$) for each congener. Differences between TEQ values from each year were compared for statistical significance ($\alpha = 0.05$) using Student's t-test with unequal variance. Normality was examined using the Shapiro-Wilk test in SigmaPlot12. Non-normal data was log-transformed and analysed to confirm results. Because log transformation did not change the interpretation of the data, all data is reported based on original (i.e., not transformed) TEQ values. All statistical tests were performed between samples collected during the same season, unless otherwise noted.

White sucker were collected from the reference site at Mountain Bay during spring and fall ($MTB_{spring} 89/91$, $MTB_{fall} 89/91$ and $MTB_{fall} 11/12$) for statistical analysis between sites. Unless otherwise noted white sucker collected from Jackfish Bay during 1989–1995 collections were compared to MTB 89/91 samples and white sucker collected in 2011 and 2012 were compared to MTB 11/12.

In order to compare TEQ values of three dated sediment cores collected from different areas of Lake Superior each section was grouped into one of five time periods. Actual dates for the mid-depth of each section from each core is available in Appendix A. For the core collected from Moberly Bay, all time periods are represented by one 1 cm section of sediment, except for “early 2000s” which represents an average of 2004 and 2005 TEQ calculations.

2.4 Results

2.4.1 White sucker

PCDD/F contamination observed in white sucker collected from Jackfish Bay declined over the period of fall 1991 to fall 2012 (Table 2.1, Fig. 2.1). The earliest analysis of PCDD/F concentrations in white sucker liver tissue occurred in 1989. There was no difference ($p = 0.628$) in mean liver TEQ collected during fall 1989 compared to 1991. In fall 1991, mean TEQ was significantly ($p = 0.006$) elevated compared to fall 1993 when ClO₂ substitution at the mill was increased from 50% to 70% at the pulp mill in Terrace Bay. PCDD/F contamination of white sucker collected from Jackfish Bay during fall continued to decline following these process upgrades. A significant ($p = 0.024$) reduction in mean TEQ, measured in white sucker liver tissue, was observed between 1993 and 1995.

White sucker captured from Sawmill Creek during spring (Table 2.2, Fig. 2.2) illustrated a similar pattern of PCDD/F contamination. The elevated mean TEQ measured in white sucker collected in 1991 appears more pronounced during spring (Fig. 2.2) compared to fall (Fig. 2.1) sampling. In contrast to fall collections, mean TEQ of white sucker collected from Jackfish Bay during the spring of 1989 was significantly ($p = 0.028$) lower compared to spring 1991. The difference in mean TEQ measured between the period of 1993 and 1995 in fish collected during the spring appears to be weaker than the difference observed in fish collected during the fall season. Lipid normalized mean TEQ of male white sucker collected from Jackfish Bay during spring (Table 2.2, Fig. 2.2) and fall (Table 2.1, Fig. 2.1) show a similar pattern to non-normalized values, reaching the highest observed level of PCDD/F contamination in 1991, followed by a decline over time. A gradual decline in PCDD/F contamination of white sucker continued to be observed in Jackfish Bay in recent years (Fig. 2.1, 2.2).

Table 2.1 PCDD/F concentrations as wet weight ($\text{pg}\cdot\text{g}^{-1}$ wet wt., mean \pm SD) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ lipid, mean \pm SD) TEQ values in liver tissue of male white sucker collected from Jackfish Bay and Mountain Bay during the fall between 1989 and 2012.

	JFB 1989	JFB 1991	JFB 1993	JFB 1995	JFB 2011	JFB 2012	MTB 1989	MTB 1991	MTB 2011	MTB 2012
2,3,7,8-TCDD	44.2 ± 19.6	52.4 ± 17.5	17.7 ± 4.83	2.70 ± 1.14	2.79 ± 1.72	1.37 ± 0.98	4.03	ND	0.73 ± 0.17	0.47 ± 0.41
1,2,3,7,8-PeCDD	ND	2.12 ± 0.64	6.38 ± 6.19	ND	0.56 ± 0.19	0.43 ± 0.11	ND	ND	0.62 ± 0.18	0.42 ± 0.19
1,2,3,4,7,8-HxCDD	ND	0.43	3.44 ± 3.30	ND	0.19 ± 0.09	0.20 ± 0.05	ND	ND	0.19	N.D
1,2,3,6,7,8-HxCDD	1.68	0.61 ± 0.11	3.05 ± 3.35	1.11	0.26 ± 0.12	0.20 ± 0.09	ND	16.5 ± 19.0	0.35 ± 0.07	0.27
1,2,3,7,8,9-HxCDD	ND	ND	3.03 ± 3.59	ND	0.18	0.15 ± 0.04	ND	ND	0.15	0.20
1,2,3,4,6,7,8-HpCDD	1.68	1.43 ± 0.21	4.56 ± 4.32	1.64 ± 0.16	0.26 ± 0.07	0.22 ± 0.07	ND	3.77	0.30 ± 0.02	0.23 ± 0.12
OCDD	120 ± 161	4.38 ± 1.02	4.67 ± 3.25	13.4 ± 10.2	0.32 ± 0.15	0.53 ± 0.53	35.54	18.9 ± 16.0	0.25 ± 0.07	0.44 ± 0.07
2,3,7,8-TCDF	285 ± 136	265 ± 111	101 ± 49.1	21.0 ± 9.32	33.6 ± 23.9	20.8 ± 17.2	20.1 ± 3.65	5.35 ± 3.16	2.53 ± 0.68	1.31 ± 0.67
1,2,3,7,8-PeCDF	ND	3.68 ± 1.20	5.34 ± 4.04	2.15 ± 1.83	0.39 ± 0.17	0.33 ± 0.15	ND	ND	0.35 ± 0.09	0.20 ± 0.10
2,3,4,7,8-PeCDF	10.4 ± 3.98	12.5 ± 2.84	5.09	ND	1.33 ± 0.69	0.75 ± 0.31	ND	1.55	0.59 ± 0.15	0.46 ± 0.26
1,2,3,4,7,8-HxCDF	ND	0.72 ± 0.16	2.75 ± 3.21	1.06	ND	0.15 ± 0.06	ND	ND	N.D	N.D
1,2,3,6,7,8-HxCDF	ND	0.29	2.53 ± 3.11	ND	ND	0.20	ND	ND	N.D	N.D
1,2,3,7,8,9-HxCDF	ND	ND	2.60 ± 3.11	ND	ND	0.16 ± 0.06	ND	ND	N.D	N.D
2,3,4,6,7,8-HxCDF	ND	0.32	2.49 ± 3.05	ND	ND	0.16 ± 0.05	ND	ND	N.D	N.D
1,2,3,4,6,7,8-HpCDF	ND	1.49 ± 0.19	4.07 ± 4.21	ND	ND	0.15 ± 0.03	ND	ND	N.D	0.22
OCDF	ND	4.63 ± 0.49	2.85 ± 3.11	ND	ND	0.21 ± 0.10	ND	ND	N.D	0.28
TOTAL TETRA-DIOXINS	44.4 ± 19.5	52.4 ± 17.5	17.7 ± 4.83	2.76 ± 1.16	3.04 ± 2.04	1.49 ± 1.15	6.59 ± 3.61	ND	1.73 ± 1.03	0.33
TOTAL PENTA-DIOXINS	ND	2.12 ± 0.64	6.38 ± 6.19	ND	0.57 ± 0.19	0.41 ± 0.13	ND	ND	0.62 ± 0.18	0.29
TOTAL HEXA-DIOXINS	1.68	0.91 ± 0.37	8.37 ± 9.97	1.11	0.30 ± 0.20	0.29 ± 0.18	ND	12.0 ± 15.6	0.83	0.47
TOTAL HEPTA-DIOXINS	2.31	1.68 ± 0.52	4.90 ± 4.02	1.64 ± 0.16	0.28 ± 0.06	0.19 ± 0.04	ND	3.77	0.30 ± 0.02	0.14
TOTAL TETRA-FURANS	285 ± 136	270 ± 108	101 ± 49.1	21.1 ± 9.32	34.2 ± 24.2	22.6 ± 18.3	20.1 ± 3.65	6.32 ± 3.57	4.84 ± 1.66	3.41 ± 3.74
TOTAL PENTA-FURANS	10.8 ± 4.22	16.8 ± 4.09	8.33 ± 4.15	2.15 ± 1.83	2.10 ± 1.32	1.33 ± 0.73	ND	1.55	2.58 ± 1.70	1.89 ± 1.80
TOTAL HEXA-FURANS	ND	1.17 ± 0.28	10.4 ± 12.5	1.06	ND	0.47 ± 0.50	ND	ND	0.32	N.D
TOTAL HEPTA-FURANS	ND	1.49 ± 0.19	4.07 ± 4.21	0.64	ND	0.28 ± 0.12	ND	ND	N.D	0.22
N	4	5 ^a	4	8	6	6	3	3	3	3
% lipid	17.8 ± 8.80	14.8 ± 4.38	67.1 ± 12.4	7.21 ± 2.63	18.9 ± 5.17	11.5 ± 5.49	17.9 ± 10.6	17.7 ± 4.15	22.4 ± 12.2	8.59 ± 2.96
TEQ^b	65.6 ± 28.0	74.29 ± 20.9	29.1 ± 11.6	4.82 ± 1.78	5.82 ± 3.42	3.34 ± 2.05	5.44 ± 1.89	1.93 ± 0.85	1.88 ± 0.45	1.06 ± 0.69
TEQ^b (lipid)	416 ± 225	563 ± 199	42.4 ± 9.42	70.8 ± 27.1	30.2 ± 13.2	27.8 ± 6.24	33.3 ± 8.14	11.9 ± 6.84	9.44 ± 3.06	11.8 ± 3.85

ND not detected; ^a Lipid normalized TEQ values calculated using n=4; ^b TEQ calculated using fish TEFs reported by Van den Berg *et al.* (1998)

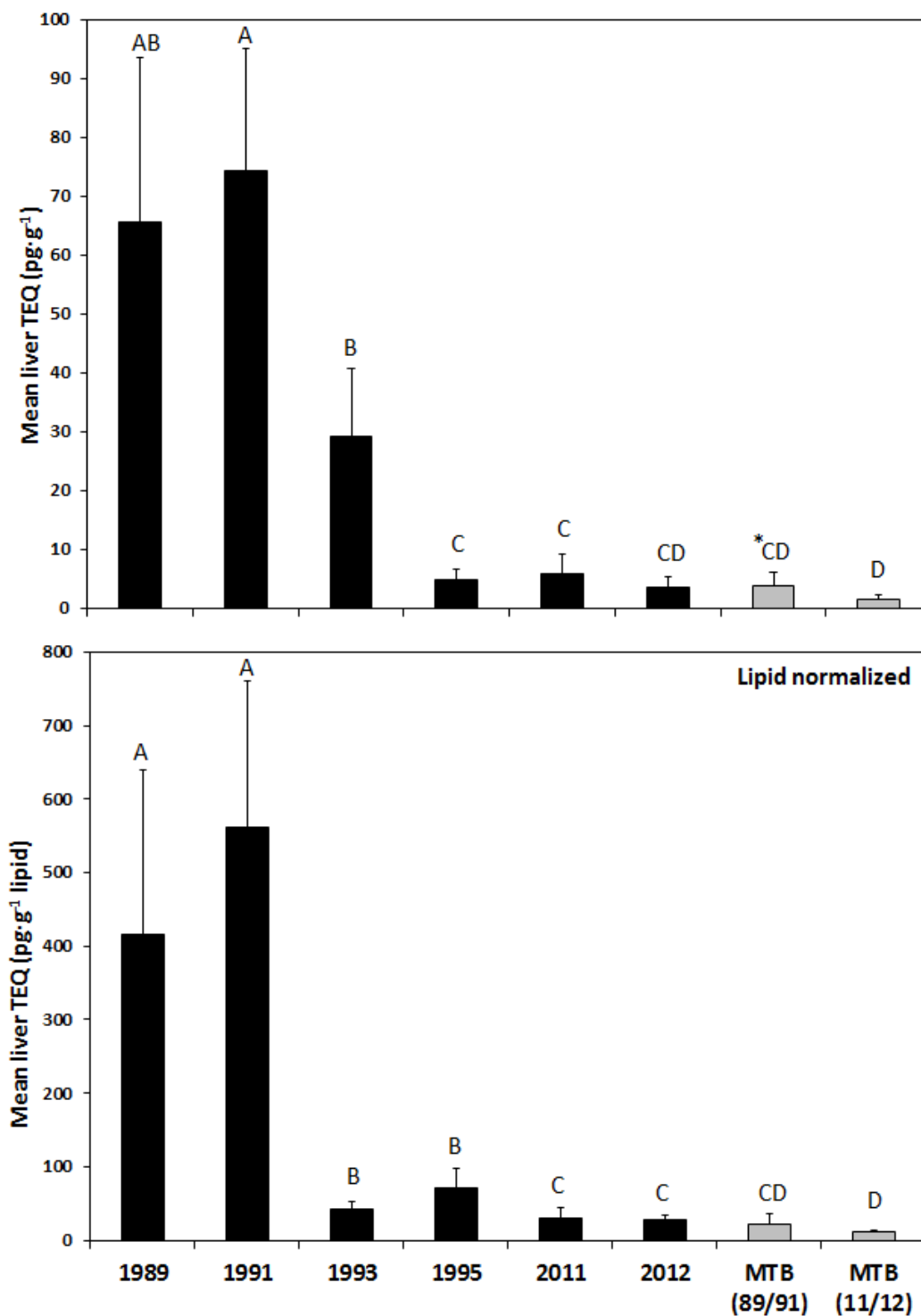


Figure 2.1 Mean liver TEQ (pg·g⁻¹ wet wt.) (top) and lipid normalized TEQ (pg·g⁻¹ lipid) (bottom) calculated using fish TEF values for liver tissue of male white sucker collected from Jackfish Bay (black) and Mountain Bay (grey) during the fall. Bars represent mean TEQ \pm SD and different letters indicate significant difference ($p < 0.05$, Student's t-test). *No statistical difference was observed between TEQ for JFB 1995 and MTB 1989 or MTB (89/91) pooled; however, JFB 1995 and MTB 1991 are different ($p < 0.05$).

Table 2.2 PCDD/F concentrations as wet weight ($\text{pg}\cdot\text{g}^{-1}$ wet wt., mean \pm SD) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ lipid, mean \pm SD) TEQ values in liver tissue of male white sucker collected from Jackfish Bay and Mountain Bay during the spring between 1989 and 2012.

	JFB 1989	JFB 1991	JFB 1993	JFB 1995	JFB 2012	MTB 1989	MTB 1991
2,3,7,8-TCDD	26.6 ± 19.1	70.8 ± 23.5	23.5 ± 16.2	3.36 ± 1.91	2.40 ± 2.88	1.20 ± 0.38	1.70 ± 0.53
1,2,3,7,8-PeCDD	1.93 ± 0.46	2.87 ± 0.46	ND	ND	0.86 ± 0.30	0.87	ND
1,2,3,4,7,8-HxCDD	0.43	ND	1.51	ND	0.22 ± 0.09	0.32 ± 0.08	ND
1,2,3,6,7,8-HxCDD	1.69 ± 0.46	2.80 ± 0.73	1.53	2.30 ± 0.15	0.35 ± 0.18	1.45 ± 0.01	1.35 ± 0.18
1,2,3,7,8,9-HxCDD	ND	ND	1.19	ND	0.170	0.63 ± 0.06	ND
1,2,3,4,6,7,8-HpCDD	1.37 ± 0.74	2.31 ± 1.00	5.58 ± 3.14	3.98 ± 2.41	0.38 ± 0.15	1.18 ± 0.40	3.07 ± 2.13
OCDD	3.62 ± 1.55	6.85 ± 3.87	14.8 ± 2.87	24.5 ± 30.0	0.40 ± 0.29	3.72 ± 0.68	4.13 ± 2.32
2,3,7,8-TCDF	202 ± 169	280 ± 111	140 ± 162	9.28 ± 4.48	32.8 ± 43.4	4.59 ± 2.67	5.34 ± 1.19
1,2,3,7,8-PeCDF	3.63 ± 1.06	5.92 ± 0.80	4.69	ND	0.51 ± 0.39	ND	ND
2,3,4,7,8-PeCDF	15.0 ± 9.31	20.2 ± 2.34	ND	4.44 ± 1.58	1.55 ± 1.25	ND	ND
1,2,3,4,7,8-HxCDF	0.96 ± 0.57	ND	0.9	1.53 ± 0.75	0.18 ± 0.05	ND	ND
1,2,3,6,7,8-HxCDF	ND	0.95	ND	ND	0.13	ND	ND
1,2,3,7,8,9-HxCDF	ND	0.95	0.58	ND	ND	ND	ND
2,3,4,6,7,8-HxCDF	ND	0.7	ND	ND	0.13	ND	ND
1,2,3,4,6,7,8-HpCDF	0.43 ± 0.31	0.80 ± 0.45	ND	4.21 ± 3.83	ND	0.27 ± 0.04	0.20 ± 0.18
OCDF	0.48 ± 0.37	1.41 ± 0.37	2.60 ± 3.87	9.38 ± 10.86	ND	0.44 ± 0.10	0.51 ± 0.12
TOTAL TETRA-DIOXINS	26.7 ± 19.0	71.7 ± 23.8	23.9 ± 15.9	3.36 ± 1.91	2.44 ± 2.98	1.69 ± 0.17	1.70 ± 0.53
TOTAL PENTA-DIOXINS	1.50 ± 0.77	2.87 ± 0.46	ND	ND	0.86 ± 0.30	0.87	ND
TOTAL HEXA-DIOXINS	1.71 ± 0.69	2.80 ± 0.73	ND	1.83 ± 0.95	0.56 ± 0.49	1.45 ± 0.01	1.47 ± 0.04
TOTAL HEPTA-DIOXINS	1.28 ± 1.05	3.14 ± 1.78	6.84 ± 3.03	4.39 ± 4.10	0.54 ± 0.31	1.47 ± 0.17	3.26 ± 1.88
TOTAL TETRA-FURANS	208 ± 168	281 ± 111	140 ± 162	9.41 ± 4.54	34.1 ± 44.2	5.41 ± 3.11	3.47 ± 3.23
TOTAL PENTA-FURANS	23.4 ± 20.1	27.6 ± 2.66	15.4 ± 11.4	3.90 ± 1.93	3.43 ± 2.47	2.94 ± 2.51	ND
TOTAL HEXA-FURANS	1.71 ± 1.84	2.60	0.83	2.17 ± 1.09	0.39 ± 0.38	ND	ND
TOTAL HEPTA-FURANS	0.43 ± 0.31	0.89 ± 0.51	ND	4.21 ± 3.83	ND	0.27 ± 0.04	0.33
N	6	4	6	16	5	3	3
% lipid	30.6 ± 9.61	40.7 ± 6.47	19.7 ± 7.90	11.3 ± 12.6	13.0 ± 7.07	32.5 ± 4.35	28.8 ± 6.58
TEQ^b	44.8 ± 31.7	97.8 ± 28.3	30.0 ± 26.7	6.22 ± 2.82	5.82 ± 6.04	2.36 ± 0.59	2.46 ± 0.97
TEQ^b (lipid)	149 ± 115	238 ± 38.1	147 ± 91.7	97.6 ± 73.0	38.7 ± 19.4	7.19 ± 0.86	8.38 ± 1.64

ND not detected; ^b TEQ calculated using fish TEFs reported by Van den Berg *et al.* (1998)

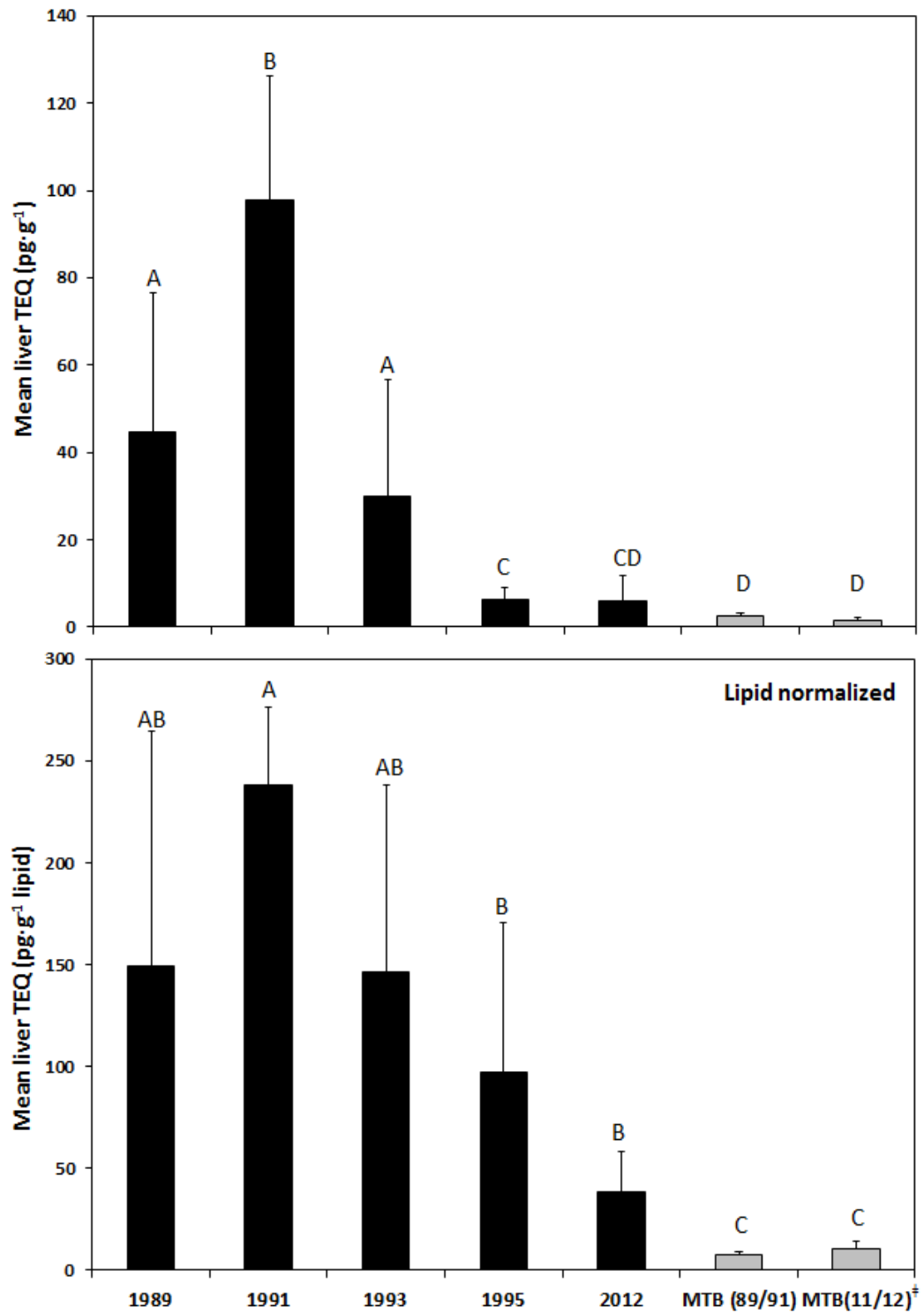


Figure 2.2 Mean liver TEQ (pg·g⁻¹ wet wt.) (top) and lipid normalized TEQ (pg·g⁻¹ lipid) (bottom) calculated using fish TEF values for liver tissue of male white sucker collected from Jackfish Bay (black) and Mountain Bay (grey) during spring. Bars represent mean TEQ \pm SD and different letters indicate significant difference ($p < 0.05$, Student's t-test). [‡] Fish collected during fall season.

Mean TEQ measured in the liver tissue of white sucker collected from Jackfish Bay (during spring and fall) between 1989 and 2012 ranged from 3.35 to 97.8 pg·g⁻¹ (wet wt.). White sucker collected from the reference site at Mountain Bay had notably lower PCDD/F contamination during spring and fall collections with mean TEQ ranging from 1.06 to 2.46 pg·g⁻¹ (wet wt.), with the exception of an elevated value of 5.44 ± 1.89 pg TEQ·g⁻¹ during fall 1989. Mean TEQ measured in white sucker collected from Mountain Bay was significantly ($p < 0.05$) lower compared to fish collected at Jackfish Bay during both fall (Fig. 2.1) and spring (Fig. 2.2) between the period of 1989 to 2011. PCDD/F contamination of white sucker in Jackfish Bay appears to be approaching background (reference) levels in recent years. In 2012 (spring and fall), when the mill was closed, mean TEQ values illustrated little (lipid normalized) or no (wet wt.) statistically significant ($p < 0.05$) difference compared to reference fish.

A distinction in contaminant profiles measured in fish and sediment was evident in Jackfish Bay, compared to Mountain Bay. The contaminant profile observed in white sucker from Jackfish Bay was consistently dominated by five congeners: 2,3,7,8-TCDD; 2,3,7,8-TCDF; 2,3,4,7,8-PCDF; 1,2,3,7,8-PeCDD; and 1,2,3,7,8-PeCDF. No difference in relative contribution to total calculated TEQ values was observed in individual congeners between 1989 and 2012. In fall 1989 and 1995 elevated concentrations of octachlorodibenzo-*p*-dioxin (OCDD) were observed in white sucker. Regardless of this, the contribution to total TEQ remained small and was not significant. On average, 2,3,7,8-TCDD and 2,3,7,8-TCDF accounted for 55% and 21%, respectively, of total calculated TEQ values between 1989 and 2012.

At Mountain Bay total TEQ calculations for white sucker liver tissue were influenced by a broader range of PCDD/F congeners, compared to Jackfish Bay. The contaminant profile of reference fish from Mountain Bay was consistently dominated by 2,3,7,8-TCDD and 1,2,3,7,8-

PeCDD, which accounted for 49–75% of total TEQ calculations during the period of 1989 to 2012. An exception to that pattern was during fall 1991 sample collections when 2,3,7,8-TCDD only comprised 9% of mean TEQ calculations.

2.4.2 Sediment

A sediment core collected from the middle of Moberly Bay illustrated an increase in TEQ values from pre-1900s until the mid to late 1980s (Fig. 1.2), followed by a drastic decline (Fig. 2.3). Once normalized for organic matter content, the distribution becomes bi-modal with two distinct peaks. The first increase in organic matter normalized TEQ was observed in approximately 1967 when values increased to $404 \text{ pg}\cdot\text{g}^{-1} \text{ OM}$. This was followed by a gradual decline until the early 1980s when TEQ values increased again, reaching levels of $533 \text{ pg}\cdot\text{g}^{-1} \text{ OM}$ around 1988. The same pattern of increasing TEQ can be observed in the core collected from the deeper water of Jackfish Bay (Fig. 2.4). TEQ values from the reference core collected at Santoy Bay remained relatively constant, with a slight peak in organic matter normalized TEQ values occurring in the late 1960s (Fig. 2.4). Organic matter normalized sediment TEQ values calculated for all years sampled from the reference core collected in Santoy Bay ranged from $9.34\text{--}23.0 \text{ pg}\cdot\text{g}^{-1} \text{ OM}$.

Overall, Moberly Bay and Jackfish Bay sediment TEQ values were consistently elevated compared to Santoy Bay. TEQ values at Moberly Bay were higher than Jackfish Bay during most years, with the exception of the 1930s–40s and early 2000s. The contaminant profile from the three core samples were consistently dominated by the same congeners: 2,3,7,8-TCDD; 1,2,3,7,8-PCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-HxCDD; 2,3,7,8-TCDF; and 2,3,4,7,8-PCDF. In the “exposed” sediment cores, 2,3,7,8-TCDF was the most influential congener to

TEQ calculations and accounted for 53% and 43% of total calculated TEQ, in Moberly Bay and Jackfish Bay, respectively. In both cores 2,3,7,8-TCDD was also an important congener, influencing 33% of total calculated TEQ. The reference core collected from Santoy Bay was dominated by 1,2,3,7,8-PeCDD (29% of TEQ) and 2,3,7,8-TCDD (17% of TEQ) with a broader range of individual congeners influencing TEQ calculations.

Surface sediment (top 2 cm) samples were collected at various distances from the mouth of Blackbird Creek to represent four areas within Jackfish Bay (Blackbird Creek, Moberly Bay, Jackfish Bay, Tunnel Bay) and two reference locations (Santoy Bay and Mountain Bay). Sediment samples from the mouth of Blackbird Creek were collected using two different methods: two mixed grab samples close to the creek where the sediment was sandy and low in organic matter, and two core samples rich in organic matter collected in the near shore area of Moberly Bay. Because the samples collected closest to Blackbird Creek represent an erosional zone, it is likely that the surface sediment (0–2 cm) represent only a few years, compared to surface sediment collected from depositional areas where the top 2 cm represent approximately 10 years.

The highest surface sediment TEQ values were observed in Moberly Bay and reached levels as high as 81.4 pg TEQ·g⁻¹. Once normalized for organic matter content, elevated TEQ values were observed throughout Jackfish Bay, including Moberly Bay, Jackfish Bay proper and Tunnel Bay (Table 2.3, Fig. 2.5). Organic matter normalized TEQ of surface sediment collected at the two reference locations outside of Jackfish Bay ranged from 30.4–50.3 pg·g⁻¹ OM, while sandy (i.e., low in organic matter) grab samples collected from Blackbird Creek reported the lowest values (Table 2.3, Fig. 2.5).

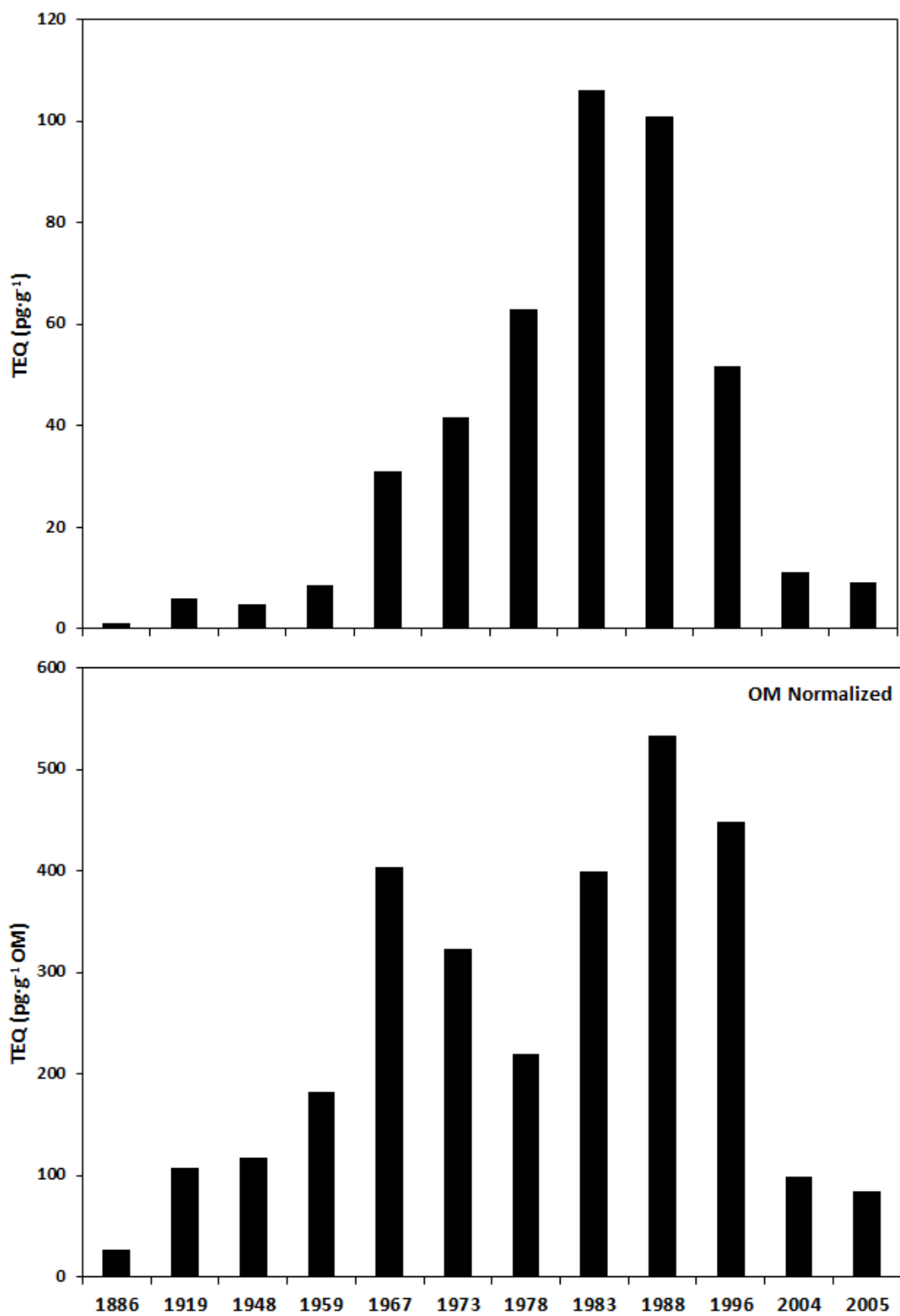


Figure 2.3 TEQ ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and organic matter (OM) normalized TEQ ($\text{pg}\cdot\text{g}^{-1}$ OM) calculated using fish TEF for a dated sediment core collected from Moberly Bay.

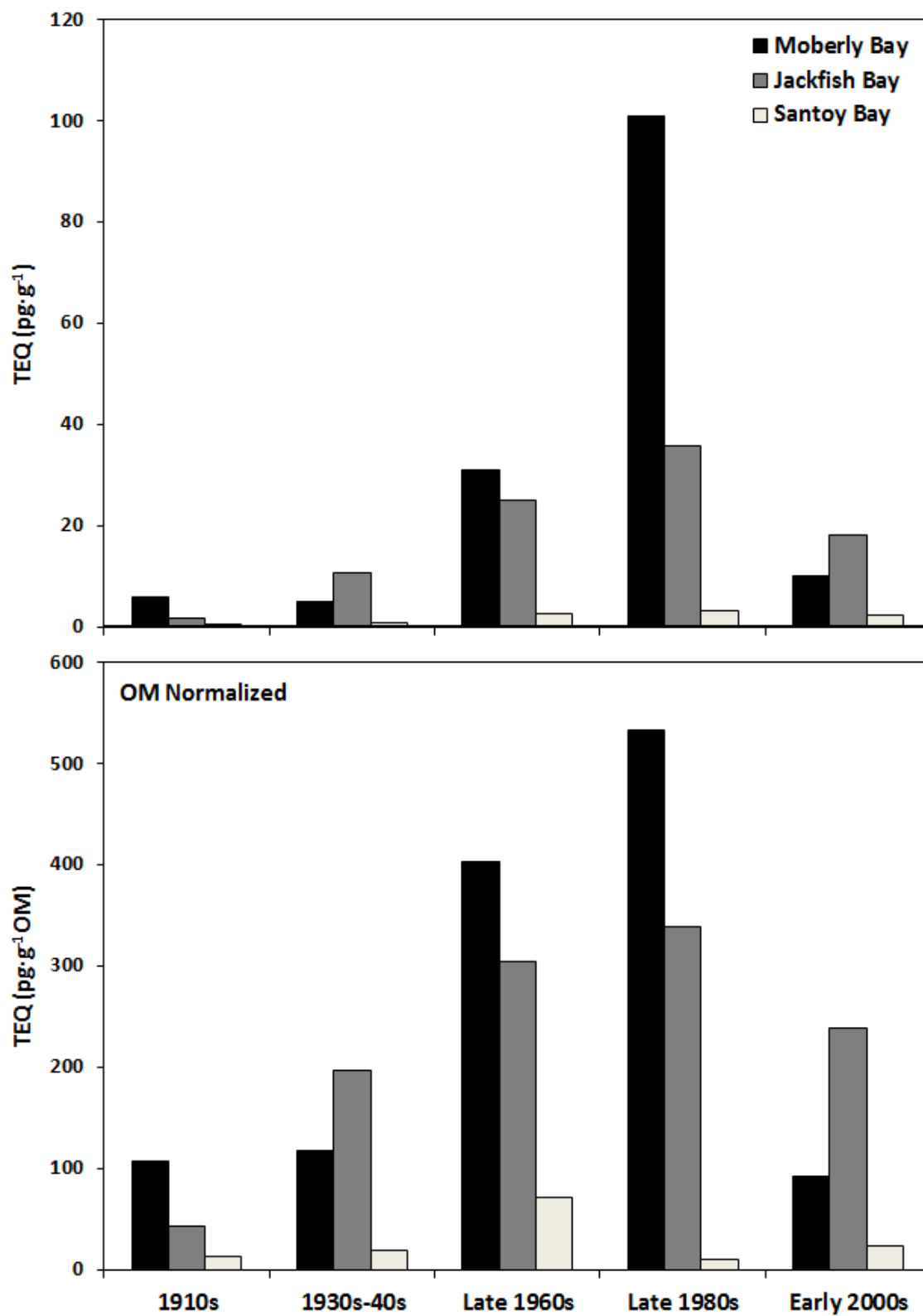


Figure 2.4 TEQ ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and organic matter (OM) normalized TEQ ($\text{pg}\cdot\text{g}^{-1}\text{ OM}$) calculated using fish TEF for dated sediment cores collected from Moberly Bay, Jackfish Bay and Santoy Bay.

The congener profile of surface sediment from all sampling locations collected was dominated by the same five congeners as previously described for the sediment cores collected in similar areas. 2,3,7,8-TCDF and 2,3,7,8-TCDD combined comprise 54–91% of calculated TEQ values for surface sediment collected from all areas of Jackfish Bay (Moberly Bay, Jackfish Bay proper and Tunnel Bay). Their influence on TEQ calculations appears to decrease with increasing distance from Moberly Bay and accounts for only 31% of calculated TEQ of surface sediment from Santoy Bay and 23% of TEQ at Mountain Bay. On the other hand, the influence of 1,2,3,7,8-PeCDD concentrations on calculated TEQ becomes greater with increasing distance from Blackbird Creek and becomes the most influential congener at the reference locations (Santoy Bay and Mountain Bay).

Table 2.3 TEQ ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and organic matter (OM) normalized TEQ ($\text{pg}\cdot\text{g}^{-1}$ OM) calculated using fish and mammalian TEFs for surface sediment collected from various distances from Blackbird Creek.

	Blackbird Creek				Moberly Bay		Jackfish Bay		Tunnel Bay		Santoy Bay		Mountain Bay	
Distance (km)	0.06	0.09	0.17	0.22	0.42	0.45	2.44	2.94	3.06	3.11	8.73	8.74	59.90	59.76
% OM	0.74	2.19	29.8	29.4	25.0	12.6	8.88	2.74	7.65	9.04	4.25	4.01	2.90	3.33
TEQ ^b	0.15	0.67	81.4	25.6	16.5	17.0	17.5	0.51	13.5	10.1	1.69	2.02	1.10	1.01
TEQ _{OM} ^b	19.6	30.5	273	87.0	65.9	135	197	18.7	177	112	39.7	50.3	37.9	30.4

^b TEQ calculated using fish TEFs reported by Van den Berg *et al.* (1998)

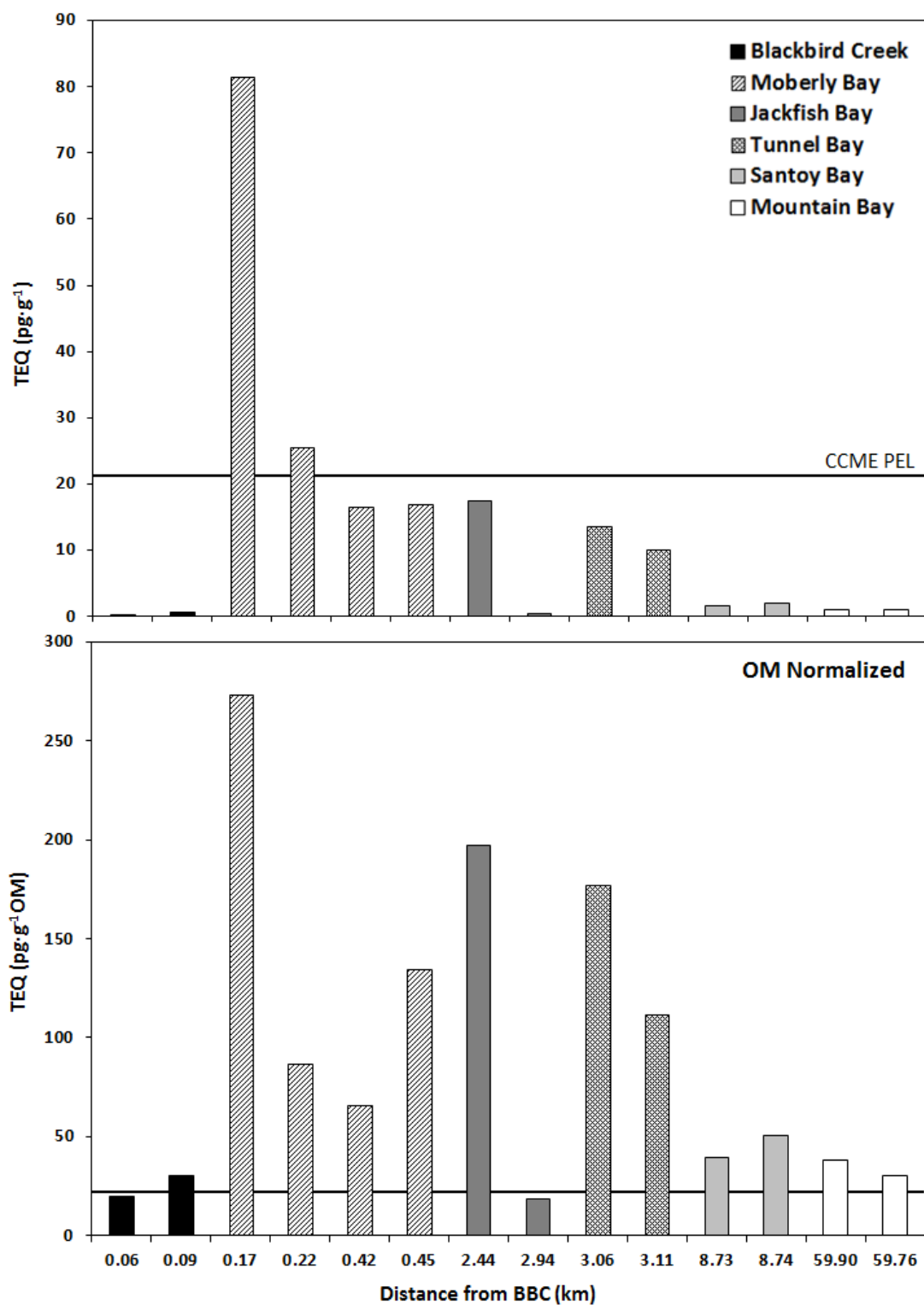


Figure 2.5 TEQ (pg·g⁻¹ dry wt.) (top) and organic matter (OM) normalized TEQ (pg·g⁻¹ OM) (bottom) calculated using fish TEF for surface sediment collected at various distances from Blackbird Creek with the CCME Probable Effects Level (PEL) shown at 21.5 pg TEQ·g⁻¹.

2.5 Discussion

Elevated PCDD/F concentrations measured at Jackfish Bay in the late 1980s and early 1990s caused concern for human consumption of fish and ecosystem health. PCDD/F contamination, however, declined dramatically in fish and sediment at Jackfish Bay following the implementation of process changes which eliminated the use of Cl_2 in the bleaching sequence. The reduction of PCDD/F TEQs in fish tissue (liver) is reflected in the contamination levels observed in dated sediment cores collected from the depositional areas of Jackfish Bay. The decline in PCDD/F contamination coincides with the timing of process changes at the pulp mill (Table 1.2, Chapter 1) which have virtually eliminated the formation of PCDD/Fs from mill effluent.

Fish collection in 1989 occurred prior to the installation of secondary treatment at the bleached kraft pulp mill in Terrace Bay (Table 1.2, Chapter 1). In addition to improved effluent treatment, the mill also increased ClO_2 substitution in the bleaching sequence to 50% in 1990 (Jackfish Bay RAP Team, 1991). No immediate decline was observed in PCDD/F contamination of fish collected from Jackfish Bay. Mean TEQ for white sucker liver tissue reached levels as high as 97.8 ± 28.2 and $74.3 \pm 20.9 \text{ pg}\cdot\text{g}^{-1}$ during spring and fall, respectively, in 1991 (Fig. 2.1, 2.2). Servos *et al.* (1994) also concluded that white sucker collected from Jackfish Bay in 1991 had elevated levels of TEQ relative to fish from reference locations and receiving environments of pulp mills that did not use chlorine bleaching. These results are consistent with many studies worldwide, which reported elevated PCDD/Fs in aquatic biota and wildlife exposed to bleached kraft mill effluent (BKME) prior to the early 1990s (Bhavsar *et al.* 2008, Elliott *et al.* 1996, Huestis *et al.* 1997, Rappe *et al.* 1987, Sandstrom *et al.* 1988, Södergren 1989, Yunker and Cretney 1996). A rapid decline in mean TEQ (wet wt.) was observed between 1991 and 1993, in

male white sucker liver collected in the spring (Table 2.2, Fig. 2.2) and fall (Table 2.1, Fig. 2.1). Lipid normalized TEQs for fish collected during the fall season also indicated a large decline. Despite the observed decline, mean TEQ values for these years remained elevated compared to reference fish collected from Mountain Bay, Lake Superior during the same time period (Fig. 2.1, 2.2).

A further decline in TEQ occurred between 1993 and 1995 (Fig. 2.1, 2.2), following an increase in the ClO₂ substitution from 50% to 70% at the pulp mill during the summer of 1993. From 1995 to present, PCDD/F contamination of white sucker from Jackfish Bay has remained relatively stable, despite the 12 month mill closure from October 2011 until October 2012. The small and varied sample size ($n = 3\text{--}16$), low concentrations and minor changes in methodology and detection limits make it difficult to compare PCDD/F contamination of white sucker between the two locations over time. However, mean TEQ of fish collected at Jackfish Bay between 1995 and 2012 appear to be approaching levels observed in reference fish from Mountain Bay. PCDD/F contamination of male white sucker collected at Jackfish Bay from 1989 to 2012 ranged from 3.34–97.8 pg TEQ·g⁻¹, compared to 1.06–5.44 pg TEQ·g⁻¹ at Mountain Bay over the same time period. The results observed in Jackfish Bay are consistent with analyses conducted on a variety of fish species across Lake Superior (Bhavsar *et al.* 2008). Bhavsar *et al.* (2008) reported a similar rapid decline between 1989 and 1993 of 2,3,7,8–TCDD; 2,3,7,8–TCDF; and 1,2,3,7,8–PeCDD concentrations in lake trout (*Salvelinus namaycush*). Recent data suggests PCDD/F contamination of the lake trout, a top predator, in Lake Superior may have reached a steady state.

A number of approaches have been used in the literature to calculate TEQ where congener concentrations are not detected during analysis. For this study a value of half the

detection limit was incorporated into TEQ calculations where congeners were reported as not detected. The high proportion of non-detectable congeners at Mountain Bay may have biased TEQ calculations for this site, particularly for historical analysis where detection limits may have been high compared to the capabilities of the current methodology. Although the use of mammalian-derived TEFs is widely accepted in the literature (Bhavsar *et al.* 2008, Huestis *et al.* 1997, Servos *et al.* 1994), they are most useful for studies investigating human health concerns. When making inferences about ecosystem health it is more informative to use fish derived TEFs (e.g., CCME 2001b). TEQ calculations reported in this study for male white sucker collected from Jackfish are based on the fish TEFs reported by the WHO (Van den Berg *et al.* 1998). Minor differences in the values reported in the current study compared to Servos *et al.* (1994, 1997) are partially due to the use of fish TEF values that place more weight on the 1,2,3,4,7,8-HxCDD; 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF congeners. This makes very little difference to the final conclusions of this study.

Different interpretations of the data can occur when TEQ calculations are normalized to lipid composition (% lipid) of individual fish. For example, no difference was observed between 1991 and 1993 in lipid normalized TEQ for male white sucker collected during the spring spawning run, nor between 1993 and 1995 male white sucker sampled during both spring and fall collections. White sucker collected at Jackfish Bay during fall 1993 exhibited unusually high (57–84%) lipid composition of liver tissue. This may have skewed the interpretation of lipid normalized TEQ measurements. A number of studies have observed disproportionate accumulation of chlorinated organic compounds in lean tissue of a variety of marine and freshwater species (Stow *et al.* 1997, Voiland *et al.* 1991, Whittle *et al.* 1993), although others have reported no significant differences (Miller *et al.* 1992). Jones *et al.* (2001) observed the

greatest proportion, by mass, of 2,3,7,8-TCDD accumulation in rainbow trout (*Oncorhynchus mykiss*) occurred in muscle tissue during gonadal recrudescence and in gonads prior to spawning. Further, accumulation of 2,3,7,8-TCDD in liver tissue appeared to reach steady-state more quickly than muscle or ovaries (Jones *et al.* 2001). Although lipid normalized TEQ of white sucker liver tissue provides additional insight into the temporal distribution of PCDD/Fs within Jackfish Bay, it is important to consider potential additional factors controlling bioaccumulation in wild fish such as food chain structure and feeding behaviour (Hebert and Keenleyside 1995, Owens *et al.* 1994, Stow *et al.* 1997, Whittle *et al.* 1993). In Jackfish Bay changes in habitat, water quality (e.g., colour, toxicity) and community structure may alter access of fish to contaminated sediment and food within different areas of the bay. The importance of feeding dynamics in PCDD/F contaminant transfer has been well documented (Kuehl *et al.* 1987, Muir *et al.* 1992, Thomann 1989, US-EPA 1993). Detrital food chain links provide the primary source of PCDD/F congeners such as OCDD which partition rapidly to surface sediments (Servos *et al.* 1989b); POM and DOM are crucial to uptake of other congeners by fish and invertebrates (Muir *et al.* 1992). Owens *et al.* (1994) attributed an observed difference in bioaccumulation of 2,3,7,8-TCDD and 2,3,7,8-TCDF between fish species to differences in feeding behavior, with higher lipid normalized concentrations found in mountain whitefish feeding on invertebrates that had ingested suspended sediments.

Reproductive responses have been measured in fish collected from Jackfish Bay for over two decades (Bowron *et al.* 2009, Munkittrick *et al.* 1994, Munkittrick *et al.* 1997). In the early 1990s investigations measured the response of fish exposed to a variety of mill effluents, with and without chlorine bleaching, and determined PCDD/F exposure in the receiving environment was not directly correlated to biological effects observed at Jackfish Bay (Servos *et al.* 1994, van

den Heuvel *et al.* 1994). PCDD/Fs were associated with a portion of MFO induction; however, it was determined that Cl_2 was not essential for induction to occur (Hodson *et al.* 1992, Nickle *et al.* 1997, Servos *et al.* 1994) and a number of the compounds contributing to induction remain unidentified (Hewitt *et al.* 2006, Kovacs *et al.* 2011). Despite this, there is still concern that environmental concentrations of PCDD/Fs below human consumption guidelines may have chronic effects on fish populations in receiving environments, contributing to the observed effects and delayed recovery. Bowron *et al.* (2009) reported a gradual recovery of white sucker over time at Jackfish Bay. Although improvements were observed in fish health, especially following conversion of the mill to ECF bleaching practices, a number of reproductive parameters remained elevated above reference condition. The recent temporary shutdowns of the Terrace Bay pulp mill demonstrated the potential for further recovery of fish in Jackfish Bay. It is unlikely that the current low concentrations of PCDD/Fs measured in this study play a role in the responses observed in fish.

To address the potential concern posed by PCDD/Fs to aquatic ecosystems, tissue residue guidelines (TRG) were developed using 1998 WHO mammalian TEF values, to protect wildlife consumers of aquatic biota (CCME 2001b). PCDD/F contamination of liver tissue from white sucker collected in Jackfish Bay exceeded the TRG ($4.75 \text{ pg TEQ} \cdot \text{g}^{-1}$) for avian consumers of aquatic biota during all sampling periods except fall 2012. It is important to note that these guidelines serve only as a framework for predicting ecosystem health. This study evaluated PCDD/F contamination in liver tissue which is high in lipid, and tends to accumulate greater amounts of PCDD/F. When considering consumption guidelines for birds and mammals, PCDD/F TEQ measured in muscle tissue (the major mass of fish and primary food source to consumers) is most informative. Because lipid normalization allows for a direct comparison

between PCDD/F contamination of different tissues (Servos *et al.* 1994), TEQ values for muscle tissue of male white sucker from Jackfish Bay would be expected to be below the TRG. The interim TRG developed for mammalian species ($0.71 \text{ pg TEQ}\cdot\text{g}^{-1}$) is lower than the TRG for avian species (CCME 2001b); however it is not likely that mammalian species would be feeding directly on white sucker.

The contaminant profile of the individual PCDD/F congeners detected in white sucker collected from Jackfish Bay was consistent throughout all sampling periods from 1989 to 2012. Five congeners consistently dominated TEQ calculations: 2,3,7,8-TCDD; 2,3,7,8-TCDF; 2,3,4,7,8-PeCDF; 1,2,3,7,8-PeCDD and 1,2,3,7,8-PCDF. This result is consistent with studies investigating PCDD/F contamination of multiple fish species throughout the Great Lakes (Bhavsar *et al.* 2008, De Vault *et al.* 1989). The Canadian National Dioxin Sampling Program, initiated in 1988, consistently identified high concentrations of 2,3,7,8-TCDD and 2,3,7,8-TCDF in aquatic organisms collected in the receiving environments of bleached kraft pulp mills that discharged into marine and freshwater environments (Whittle *et al.* 1993). Differences in relative proportion of 2,3,7,8-TCDD: 2,3,7,8-TCDF have been identified in fish collected from contaminated sites in the upper and lower Great Lakes, indicating different sources of pollution (De Vault *et al.* 1989, Huestis *et al.* 1997, Whittle *et al.* 1992). Increased proportions of 2,3,7,8-TCDF compared to 2,3,7,8-TCDD have been measured in fish collected from Lake Superior, suggesting the source of contamination originates from the historical BKME discharge with significant localized sources in various areas of the lake, including Jackfish Bay (Servos *et al.* 1994, Whittle *et al.* 1992). Throughout the time period of this study the proportion of 2,3,7,8-TCDD: 2,3,7,8-TCDF gradually increased in fish collected from Jackfish Bay. At the same time the relative contribution of individual congeners to TEQ evolved to partially resemble the

contaminant profile observed in white sucker collected from Mountain Bay. For example, the gradual increase in proportion of 2,3,7,8-TCDD observed at Jackfish Bay in recent years reflects contamination seen in reference fish from Mountain Bay. However, white sucker from Jackfish Bay continue to show elevated levels of 2,3,7,8-TCDF relative to reference fish (Table 2.1, 2.2). The contaminant profile observed in the sediment samples differed from that of white sucker, with a greater emphasis on penta (PeCDD/F) and hexa (HxCDD/F) congeners. However 2,3,7,8-TCDF and 2,3,7,8-TCDD remain the most influential congeners to the calculated TEQ values in sediment collected from all areas of Jackfish Bay.

Analysis of a sediment core collected from the depositional area of Moberly Bay illustrates increasing concentrations of PCDD/Fs, particularly 2,3,7,8-TCDF, from approximately the late 1960s to the late 1980s or early 90s. This corresponds to a period of mill expansion which included the introduction of a fully bleached ‘hot’ kraft process (Jackfish Bay RAP Team, 1991). Because molecular chlorine is less soluble at higher temperatures this had the potential to affect by-product formation (Smook 1992). In addition, the use of oil-based defoamers in brown stock washers may have provided precursors for 2,3,7,8-TCDF formation (Allen *et al.* 1989, Sherman *et al.* 1990). These results are consistent with the trends observed in a dated sediment core from Moberly Bay collected by Sherman *et al.* (1990) in 1988.

The rapid decline of TEQ observed in white sucker collected from Jackfish Bay following an increase in ClO₂ substitution to 50% in 1990 suggests that uptake of PCDD/Fs by fish was minimal, despite the physical-chemical properties which allow PCDD/Fs to persist in sediment with the potential for bioaccumulation. A similar decline in PCDD/F contamination was observed in a dated sediment core from Moberly Bay (Fig. 2.3). Sediment TEQ values peaked in the mid-to-late 1980s, followed by a rapid decline. The decline in sediment TEQ

corresponds to implementation of a number of process changes at the mill (Table 1.2, Chapter 1) which resulted in a reduction of PCDD/Fs in mill effluent. These upgrades included increased ClO_2 substitution to 70% and upgraded ClO_2 generators, which allowed the mill to continually produce ECF pulp by eliminating the production of chlorine by-products. A further decline was observed in sediment TEQ following a shift to 100% ClO_2 substitution in 1998 (mill no. 1) and 1999 (mill no. 2). It was been well demonstrated that a dramatic reduction of 2,3,7,8-TCDD and 2,3,7,8-TCDF formation, to non-measurable levels, can occur following an increase to 70% or greater ClO_2 substitution in the first stage of the bleaching process (Parthasarthy *et al.* 1994).

Despite removal of PCDD/F precursors and Cl_2 from the bleaching sequence, their persistence may allow PCDD/Fs to remain bioavailable in surface sediment for long periods of time. Experimental determination of depuration rates in liver tissue of rainbow trout fed 1.8 and 18 $\text{pg}\cdot\text{g}^{-1}$ 2,3,7,8-TCDD suggest that following removal of the source of contamination the tissue concentration will decrease by approximately half every 40 d (Jones *et al.* 2001). These results suggest 2,3,7,8-TCDD would be virtually eliminated from white sucker in Jackfish Bay within one year of complete removal of the source of PCDD/Fs. This is consistent with the decline in PCDD/F TEQ observed in white sucker following mill process changes to remove chlorine from the bleaching sequence. Many studies have determined the bioaccumulation of PCDD/Fs such as 2,3,7,8-TCDD in fish is not linked to surface sediment contamination and that uptake of highly chlorinated PCDD/Fs occurs predominantly through POM and DOM via food chain transfer (Foster *et al.* 1999, Muir *et al.* 1992, Owens *et al.* 1994, Servos *et al.* 1992b). Servos *et al.* (1992c) showed a shift in the 1,3,6,8-TCDD uptake route in mesocosms over time, transitioning from initial equilibrium partitioning in the water column to detrital food chain transfer. In

Moberly Bay, Sherman *et al.* (1990) were able to show that suspended solids in mill effluent, not surficial sediment, were the primary source of 2,3,7,8-TCDF.

Sherman *et al.* (1990) suggested that OCDD concentrations in aquatic organisms collected from Moberly Bay were not related to mill effluent and likely accumulated through detrital food chains. This is consistent with evidence that OCDD partitions rapidly to sediment (Servos *et al.* 1989a). Many studies have identified a unique “bleaching pattern” of highly chlorinated PCDD/F congeners, distinct from the pattern produced by air emissions. The incineration pattern of PCDD/Fs has been determined to include hepta (HpDD) and octa (OCDD) dioxin congeners as well as 1,3,6,8- and 1,3,7,9-TCDFs and penta (PeCDF) furans (Rappe *et al.* 1989, Swanson *et al.* 1988). The congener pattern produced by bleached kraft pulp mills is dominated by 2,3,7,8-TCDD and 2,3,7,8-TCDF formed during the chlorination and extraction processes due to the presence of precursors and the use of Cl₂ (Swanson *et al.* 1988). High concentrations of OCDD observed in white sucker from Jackfish Bay (Table 2.1, 2.2) and sediment cores from Moberly Bay (Table A.2) do not provide a meaningful contribution to total TEQ calculations and can likely be attributed to emission sources or the use of treated wood. Pentachlorophenol and creosote used to treat wood products have been shown to contain high levels of highly chlorinated PCDD/Fs, including OCDD (Dougherty *et al.* 1978, McKee *et al.* 1990). The use of treated wood likely resulted in contamination of mill effluent at some period of time during the history of the mill operation. For example, a 250 m section of creosote treated wooden stave pipe was replaced in 1994 (Table 1.2, Chapter 1). According to Pearson *et al.* (1998), the current PCDD/F load received by Lake Superior is almost entirely due to atmospheric sources.

Moderate contamination of surface sediments (0–2 cm) was found throughout Jackfish Bay (Table 2.3, Fig. 2.5). PCDD/Fs of two surface sediment samples collected in the near shore depositional area of Moberly Bay exceeded the CCME probable effects level (PEL) (21.5 pg TEQ·g⁻¹), developed using fish TEFs (Van den Berg *et al.* 1998) to evaluate potential for PCDD/F exposure to cause adverse biological effects (CCME 2001a). This is consistent with the pattern observed for total organic carbon which was highest in the depositional area of Moberly Bay and decreased with distance from the mouth of Blackbird Creek (Table 2.3). Surface sediment samples collected closest to the mouth of Blackbird Creek represent an erosional area with low organic matter. Surface sediment from this area likely represents only a few years with sediment being removed quickly and transported to the depositional areas of Moberly Bay or exported to Lake Superior. The sediment core collected from the further depositional area of Jackfish Bay, near St. Patrick Island, illustrates TEQ levels below the PEL in the top 2 cm (representing approximately 10 years). Below this level the PEL was exceeded with TEQ ranging from 31.0–106 pg·g⁻¹ between 1967 and 1996 (Table 2.3, Fig. 2.5). During the same time period the core from Jackfish Bay also exceeded the PEL, reaching a maximum TEQ of 35.9 pg·g⁻¹. Sediment samples collected from Tunnel Bay illustrated elevated TEQ compared to reference sediment but remained below the PEL. These results are consistent with benthic community collections within Jackfish Bay, indicating a polluted environment within Moberly Bay (Milani 2009). Milani (2009) also found the benthic communities outside of Moberly Bay were similar to those observed in reference locations, with some differences in the densities of tubificids and amphipods.

PCDD/F concentrations have been declining in fish and sediment from Jackfish Bay since the early 1990s. This study provides evidence that the decrease in contamination is

associated with mill process changes which resulted in a reduction of the use of Cl_2 in the bleaching sequence. Current PCDD/F concentrations measured in white sucker from Jackfish Bay are below TRG levels and therefore likely not indicative of impaired ecosystem health. High PCDD/F concentrations remaining in surface sediments throughout Jackfish Bay do not appear to be translated to fish. POM and DOM from mill effluent were likely the primary source of contamination to fish through food chain transfer from invertebrates ingesting sediment.

Chapter 3 Historical trends of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in white sucker (*Catostomus commersoni*) from the Mattagami River, following the closure of the bleached kraft pulp mill at Smooth Rock Falls

Contributors to this chapter are:

Cater, Shari C.¹, Tim J. Arciszewski², Mark E. McMaster³, Kelly R. Munkittrick² and Mark R. Servos¹

1 Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1

2 Department of Biology, University of New Brunswick, Saint John, NB E2L 4L5

3 Ecosystem Health Assessment, Environment Canada, Burlington, ON L7R 4A6

- Shari Cater: M.Sc. candidate who researched, collected, analyzed and wrote the paper
- Tim Arciszewski: Organized and assisted with fish collection
- Kelly Munkittrick: Assisted with field work, past and present, and provided general advice
- Mark McMaster: Committee member of Shari Cater. Assisted with field work, past and present, and provided general advice
- Mark Servos: Supervisor of Shari Cater. Collected historical data, assisted with field work, ideas, research direction, editing, and provided general advice.

3.1 Summary

In the early 1990s worldwide concern arose regarding the formation of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) produced by bleached kraft pulp mills utilizing molecular chlorine (Cl₂) in their bleaching process. In response to the growing concern, scientists initiated a study to evaluate the relationship between environmental response and various mill processes used across Ontario. The study measured the biological effects and PCDD/F contamination of white sucker (*Catostomus commersoni*) collected from the receiving environment of several pulp mills. The highest PCDD/F concentrations were measured downstream of the pulp mill in Smooth Rock Falls which employed primary effluent treatment and Cl₂ bleaching. Mean toxic equivalents (TEQ) measured in liver tissue of white sucker exposed to the bleached kraft mill effluent (BKME) from the Smooth Rock Falls mill were reported as high as 111 ± 86.2 pg TEQ·g⁻¹. These results contributed to the implementation of new effluent regulations and a national pulp and paper Environment Effects Monitoring (EEM) program in Canada which led to an industry shift toward elemental (ECF) and total chlorine free (TCF) bleaching practices. By 1992 the mill in Smooth Rock Falls had upgraded to 100% chlorine dioxide (ClO₂) substitution and eliminated PCDD/Fs from its effluent. This study examines temporal changes in the tissue burden of white sucker (measured as PCDD/F TEQ) following these process upgrades and subsequent closure of the mill in 2006. A rapid decline in PCDD/F contamination in liver tissue was observed following the process upgrades put in place to comply with new regulations. Mean TEQ measured in 1993 and 1995 were approaching levels observed in reference fish. In recent years (2011 and 2012) a return to background condition (0.53–1.49 pg TEQ·g⁻¹) was observed in white sucker collected downstream of the historical mill outfall with PCDD/F contamination ranging from 0.75–2.87 pg TEQ·g⁻¹. Trends were also

observed in the PCDD/F contaminant pattern of “exposed” fish. Contribution of 2,3,7,8–TCDD and 2,3,7,8–TCDF to total TEQ declined dramatically in recent years and was more representative of reference fish compared to white sucker collected downstream in 1991. The rapid recovery of PCDD/F contamination of white sucker downstream of the Smooth Rock Falls pulp mill observed in this study are attributed to the process upgrades and closure of the mill as well as the removal of contaminated suspended solids by the fast flowing nature of the Mattagami River.

3.2 Introduction

Effluents from pulp mills using molecular chlorine (Cl_2) in their bleaching sequence have been associated with contamination and adverse effects in aquatic environments (McMaster *et al.* 1991, Munkittrick *et al.* 1991, 1994, Södergren 1989). Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are toxic organic pollutants produced by the bleaching process in pulp and paper manufacturing. The formation of PCDD/Fs in bleached kraft mill effluent (BKME) has been attributed to the use of oil-based defoamers and brown stock washers containing PCDD/F precursors, in combination with the use of Cl_2 in the chlorination stage of the bleaching sequence (Amendola *et al.* 1989, Axegård and Renberg 1989, Berry *et al.* 1989, Swanson *et al.* 1988). In the late 1980s and early 1990s high concentrations of PCDD/Fs were measured in water, sediment and biota from several BKME receiving environments in Canada (Servos *et al.* 1994, Sherman *et al.* 1990).

The pulp mill in Smooth Rock Falls, on the Mattagami River in northern Ontario, was converted to a bleached kraft process in 1927. The mill underwent a number of process changes and treatment upgrades during operation, including installation of primary (1976) and secondary

(1994–5) effluent treatment (BEAK 1998). Reproductive responses were reported in white sucker (*Catostomus commersoni*) collected in the BKME receiving environment of the mill during the early 1990s. This included increased condition, reduced gonad size, increased liver size, reduced circulating sex steroid levels, reduced vitellogenin in females and elevated microsomal mixed-function oxidation (MFO) activity (ESG 1998, 2000, Munkittrick *et al.* 1994, Munkittrick *et al.* 2000, Nickle *et al.* 1997, Stantec 2004a, 2007). During the same time period very high PPCD/F concentrations were reported in muscle and liver tissue of white sucker collected downstream of the mill (Servos *et al.* 1994)

The high PCDD/F concentrations and biological effects observed in fish residing in the receiving environment of many mills contributed to the implementation of new Pulp and Paper Effluent Regulations and a national Environmental Effects Monitoring (EEM) program for pulp and paper mills. The new regulations under the Canadian Environmental Protection Act (CEPA) required non-measurable levels of PCDD/Fs in mill effluents by January 1994 (CEPA 1999). This was accomplished by upgrading bleaching processes to elemental chlorine free (ECF) technologies, including increased chlorine dioxide (ClO₂) substitution (Luthe 1998, McDonough 1995). The pulp mill in Smooth Rock Falls installed ClO₂ generators in 1991 and increased ClO₂ substitution from 16 to 100% by 1992 to comply with new regulations (Table 1.3, Chapter 1).

In 2006 the pulp mill in Smooth Rock Falls was permanently shut down. Despite the virtual elimination of PCDD/Fs from mill effluent and subsequent mill closure, PCDD/Fs provide a potential source of long term contamination to aquatic ecosystems due to their long half-lives in sediment and fish (Hahn 2001, Jones *et al.* 2001, Parrott *et al.* 1995, Segstro *et al.* 1995). It was expected that high levels of PCDD/Fs may remain in surface sediment within the receiving environment following their removal from mill effluents and would be bioavailable to

aquatic biota for many years. The lipophilic nature of PCDD/Fs allows them to bioaccumulate in biota (Muir and Servos 1996). This has caused concern for the health of aquatic ecosystems and potential implications for human consumption guidelines of fish residing in areas historically exposed to BKME. There have been very few studies that have documented the long term persistence of PCDD/Fs in fish following their removal from effluents. Early studies investigating the effects of pulp mill effluents and contamination of PCDD/Fs in the receiving environment of the Mattagami River provide an opportunity to investigate the legacy of PCDD/F contamination from historical BKME discharges from the mill at Smooth Rock Falls.

The objective of this study was to determine how PCDD/F contamination of white sucker in the Mattagami River changed following a decade of process changes and subsequent closure of the bleached kraft pulp mill in Smooth Rock Falls, Ontario. PCDD/F concentrations were measured in liver tissue of white sucker collected upstream (reference) and downstream (“exposed”) of the historical effluent discharge during fall 2011 and 2012. These were compared to historical PCDD/F contamination measured in white sucker collected downstream in the early 1990s.

3.3 Materials and Methods

3.3.1 Study site

The Mattagami River is a major tributary of the Moose River basin in northeastern Ontario. It contains hydroelectric dams, water storage reservoirs and historically (1918–2006) contained a bleached kraft pulp mill in the town of Smooth Rock Falls (Munkittrick *et al.* 2000). The pulp mill at Smooth Rock Falls was located immediately below a run-of-the river dam which prevented fish from migrating upstream. The Mattagami River also contains a set of

rapids, Cypress Falls, approximately 30 km downstream of the mill. Further downstream on the Mattagami River, beyond the confluence with the Kapuskasing River (which contains a thermo-mechanical pulp mill at the town of Kapuskasing), there are four dams and associated head ponds: Little Long, Smoky Falls, Harmon and Kipling. While in operation, effluent discharged from the Smooth Rock Falls pulp mill remained at > 1% dilution for up to 64 km downstream of the mill with foam observed up to 5 km downstream (ACRES 1996, Brousseau and Goodchild 1989, Munkittrick *et al.* 2000).

Upstream of the dam at Smooth Rock Falls the reservoir forms a deep depositional area with substrate dominated by organic matter, silt and clay. Immediately downstream of Smooth Rock Falls the substrate is primarily sand with maximum depths of approximately 5 m. Further downstream, below Cypress Falls, the substrate becomes dominated by clay. No depositional areas were found on the Mattagami River between Smooth Rock Falls and the Little Long dam; therefore, no sediment core samples were available for PCDD/F analysis at this site.

3.3.2 Fish collection and sampling procedures

Three sites were sampled on the Mattagami River (Fig. 1.5, Chapter 1): a reference site 3–5 km upstream of the dam at Smooth Rock Falls, an exposure site immediately (0.5–1 km) downstream of the historical location of mill effluent discharge and a second exposure (and possible recovery) site at Cypress Falls. Collection of white sucker (20 male and 20 female from each site) on the Mattagami River occurred in September 2011 and September 2012 using gill nets with 8.8 and 10.2 cm (3.5 and 4 inch) mesh sizes. A subset of 3–6 white sucker liver tissue samples were randomly selected from each site for PCDD/F analysis. Fish were collected and processed as described in Chapter 2.

3.3.3 PCDD/F analysis

PCDD/F analysis was conducted by AXYS Analytical Services Ltd. (Sidney, BC) as described in Chapter 2.

3.3.4 Statistical analysis

The statistical approach used for this study is described in Chapter 2. Briefly, toxic equivalents (TEQ) were calculated using congener specific fish toxic equivalency factors (TEF) reported by the World Health Organization (Van den Berg *et al.* 1998). TEQ values for tissue samples are reported as wet weight ($\text{pg}\cdot\text{g}^{-1}$ wet wt.) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ lipid) values. Where PCDD/F concentrations were below detection limits (reported as ND), a value of one half of the sample detection limit (DL) was used to calculate TEQ. Differences between years was assessed for statistical significance ($\alpha = 0.05$) using Student's t-test with unequal variance. Normality was examined using the Shapiro-Wilk test in SigmaPlot12. Non-normal data was log-transformed and analysed to confirm results. Because log transformation did not change the interpretation of the data, all data are reported based on original (i.e., not transformed) values.

3.4 Results

A dramatic decline was observed in PCDD/F contamination of white sucker downstream of the pulp mill at Smooth Rock Falls between 1991 and 2011/12. In 1991 mean TEQ measured in liver tissue of white sucker exposed to BKME downstream was significantly ($p = 0.009$) elevated compared to fish collected downstream in 2011 (Table 3.1, Fig. 3.1), five years after the pulp mill was permanently shut down (Table 1.3, Chapter 1). White sucker collected further downstream at Cypress Falls in 2012 also illustrated significantly ($p = 0.009$) lower mean liver TEQ compared to 1991. There was no difference ($p > 0.05$) in PCDD/F contamination between

fish collected immediately downstream in 2011 and those collected at Cypress Falls in 2012. This pattern was consistent among lipid normalized mean TEQ during the same time period (Table 3.1, Fig. 3.1). Lipid content of white sucker collected in the receiving environment of the mill in 1991 was significantly ($p < 0.001$) higher compared to fish collected upstream and downstream in 2011 and 2012.

No difference in mean TEQ of reference fish collected upstream of the dam at Smooth Rock Falls was observed between 2011 and 2012; therefore, samples were pooled for analysis to increase statistical power. Mean TEQ measured in reference fish (2011 and 2012) was significantly ($p = 0.009$) lower compared to exposed fish collected downstream of the mill in 1991. PCDD/F contamination of white sucker collected immediately downstream in 2011 was not different ($p > 0.05$) compared to reference fish collected upstream in 2011 and 2012. In 2012 mean TEQ measured in fish collected at Cypress Falls exhibited a small difference ($p = 0.038$) compared to reference fish collected in 2012 and no difference ($p = 0.1$) compared to fish collected upstream in 2011. Because of the small sample size and potential bias in reference sample selection in 2012, the difference was not considered to be meaningful (Fig. 3.1).

Once lipid normalized a statistically significant ($p < 0.05$) difference was observed between reference fish in 2011 and 2012 compared to white sucker collected downstream during all sampling periods. The difference observed in lipid normalized TEQ measured in liver tissue between fish collected downstream in 2011 and 2012 compared to pooled reference fish (MAT-UP 11/12) is strongly influenced by the 2012 reference fish. Although no difference exists in the lipid content of the fish, the difference in PCDD/F contamination could be driven by a potential sampling bias (e.g., fish age).

Mammalian TEQ values were calculated for this data set using international TEF (I-TEF) values (Kuntz et al., 1990; Table B.1, Appendix B) in order to allow a direct comparison to TEQ reported in white sucker collected downstream of Smooth Rock Falls in 1993 and 1995 as reported by Servos *et al.* (1997). TEQ values for white sucker collected in 1991, reported in both studies, originate from the data set presented by Servos *et al.* (1994). Lipid normalized TEQ values were calculated using the mean lipid content of fish (rather than individual % lipid) collected during each time period due to the availability of historical data. For comparison purposes lipid normalized mammalian TEQ values for white sucker collected in this study were reported in the same manner. This comparison (Fig. 3.2) illustrates a rapid decline in mean TEQ between 1991 and 1993. In contrast to this trend a small increase in TEQ was observed downstream during 1995 before declining to levels at or near background values in 2011 and 2012. Overall, mean TEQ values of white sucker liver tissue measured during 1993 and 1995 appear to be approaching reference levels observed upstream in 1995, although mean TEQ remained elevated compared to reference fish collected in 2011 and 2012. A similar decline was observed in lipid content over this time period (1991–2012). Elevated TEQ values were reported for reference fish collected upstream in 1995 compared to 2011 and 2012 despite a mean lipid content of approximately 14% reported for all three collection periods. One possible explanation for this may be the presence of an outlier in the 1995 data set, as suggested by the larger than expected standard deviation.

Table 3.1 PCDD/F concentrations as wet weight ($\text{pg}\cdot\text{g}^{-1}$ wet wt., mean \pm SD) and lipid normalized ($\text{pg}\cdot\text{g}^{-1}$ lipid, mean \pm SD) TEQ values in liver tissue of male white sucker collected in the fall upstream (MAT-UP), 1 km (MAT-DN) and 30 km (MAT-CY) downstream of the pulp mill at Smooth Rock Falls between 1991 and 2012.

	MAT-UP		MAT-DN		MAT-CY
	Fall 2011	Fall 2012	Fall 1991	Fall 2011	Fall 2012
2,3,7,8-TCDD	0.57 ± 0.11	0.36 ± 0.11	83.7 ± 63.7	0.54 ± 0.18	0.96 ± 0.36
1,2,3,7,8-PeCDD	0.31 ± 0.03	0.24 ± 0.11	4.81 ± 3.29	0.51	0.22 ± 0.05
1,2,3,4,7,8-HxCDD	ND	0.12	ND	0.46 ± 0.02	0.24
1,2,3,6,7,8-HxCDD	0.65 ± 0.19	0.43 ± 0.10	1.13 ± 0.88	0.62 ± 0.22	0.42 ± 0.17
1,2,3,7,8,9-HxCDD	2.06 ± 1.84	0.35 ± 0.08	ND	0.85 ± 0.28	0.55 ± 0.30
1,2,3,4,6,7,8-HpCDD	ND	0.13	3.11 ± 1.23	ND	0.35 ± 0.17
OCDD	ND	ND	5.51 ± 1.41	0.41	0.20 ± 0.06
2,3,7,8-TCDF	ND	ND	309 ± 253	0.37	0.21
1,2,3,7,8-PeCDF	0.25	ND	4.00 ± 3.76	ND	0.23
2,3,4,7,8-PeCDF	ND	ND	15.5 ± 14.6	0.52	0.24
1,2,3,4,7,8-HxCDF	0.35	ND	0.69	0.46	0.36 ± 0.21
1,2,3,6,7,8-HxCDF	0.64 ± 0.04	0.41 ± 0.13	0.31	0.73 ± 0.47	0.52 ± 0.17
1,2,3,7,8,9-HxCDF	0.57	0.52 ± 0.15	1.41	ND	0.40 ± 0.31
2,3,4,6,7,8-HxCDF	1.30	0.52 ± 0.24	ND	0.47	1.88 ± 1.13
1,2,3,4,6,7,8-HpCDF	1.78 ± 0.37	2.52 ± 1.90	1.62 ± 1.49	3.61 ± 0.96	22.7 ± 13.3
OCDF	ND	ND	2.33 ± 2.13	0.52	0.24
TOTAL TETRA-DIOXINS	0.64 ± 0.04	0.38 ± 0.10	83.7 ± 63.7	0.52 ± 0.20	0.42 ± 0.18
TOTAL PENTA-DIOXINS	0.46 ± 0.15	0.41 ± 0.19	4.81 ± 3.29	0.45 ± 0.08	0.31 ± 0.16
TOTAL HEXA-DIOXINS	1.97 ± 1.29	1.97 ± 0.78	1.13 ± 0.88	3.38 ± 0.76	18.4 ± 8.29
TOTAL HEPTA-DIOXINS	0.77 ± 0.20	0.53 ± 0.20	3.31 ± 1.27	0.57 ± 0.24	0.68 ± 0.19
TOTAL TETRA-FURANS	ND	ND	309 ± 253	ND	ND
TOTAL PENTA-FURANS	ND	ND	20.2 ± 18.9	0.38	ND
TOTAL HEXA-FURANS	0.79 ± 0.29	0.45 ± 0.13	1.41	0.83 ± 0.37	0.43 ± 0.25
TOTAL HEPTA-FURANS	ND	0.18	1.62 ± 1.49	0.78	0.59 ± 0.62
N	3	3	8	6	6
% Lipid	14.6 ± 5.73	14.3 ± 2.99	46.8 ± 10.7	9.03 ± 3.10	12.5 ± 5.56
TEQ	1.20 ± 0.27	0.80 ± 0.31	111 ± 86.2	1.17 ± 0.51	1.83 ± 0.73
TEQ _(lipid)	8.94 ± 3.01	5.49 ± 1.04	237 ± 163	13.6 ± 5.08	15.5 ± 4.05

ND not detected; ^b TEQ calculated using fish TEFs reported by Van den Berg *et al.* (1998)

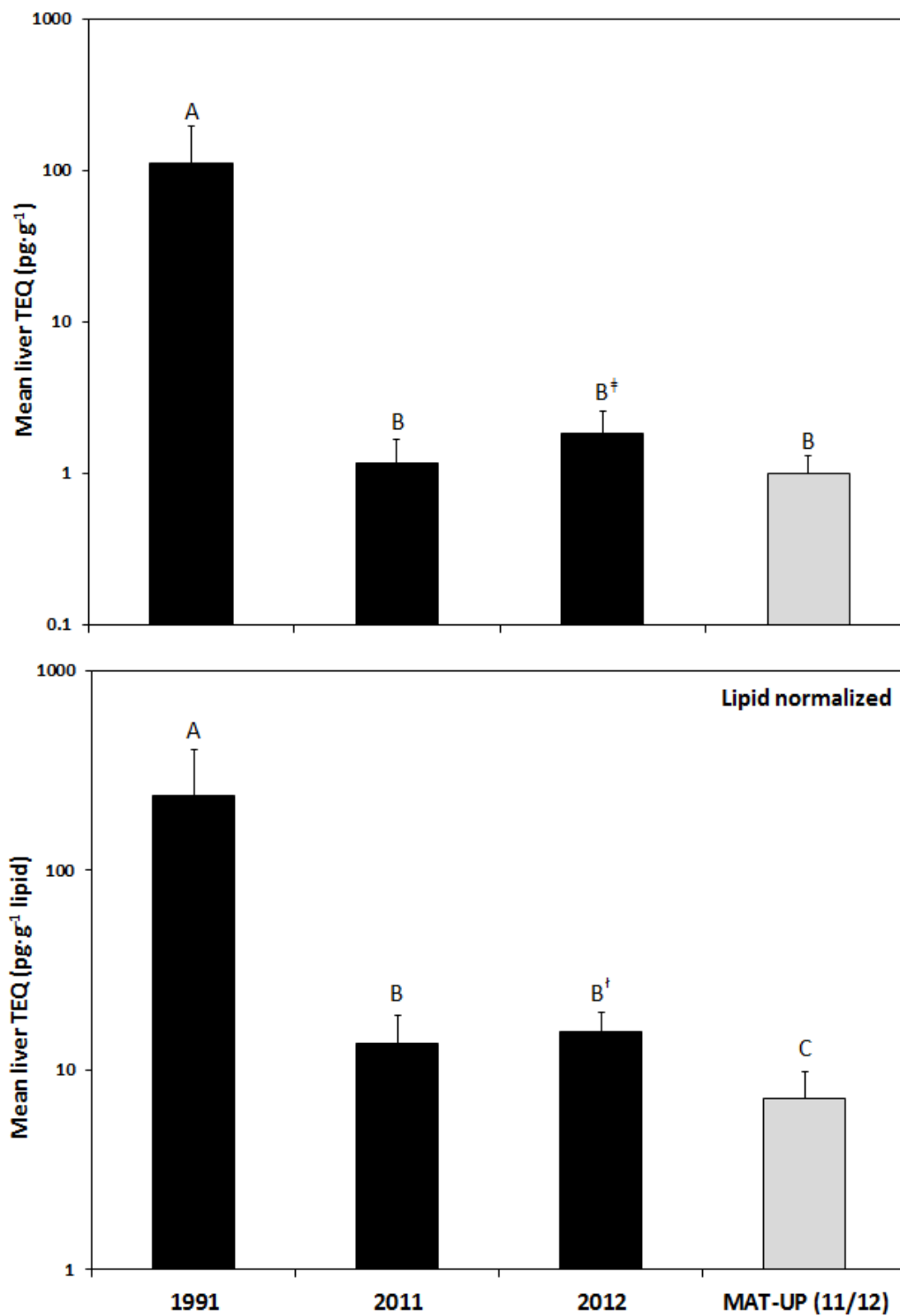


Figure 3.1 Mean liver TEQ (pg·g⁻¹ wet wt.) (top) and lipid normalized TEQ (pg·g⁻¹ lipid) (bottom) calculated using fish TEF values for liver tissue of male white sucker collected upstream (grey) and downstream (black) of the pulp mill at Smooth Rock Falls. Bars represent mean TEQ ± SD and different letters indicate significant difference (p < 0.05, Student's t-test). ‡ No diff. vs. MAT-UP 2011 but sig. diff. vs. MAT-UP 2012. † Small diff. vs. MAT-UP 2011, larger diff. vs. MAT-UP 2012.

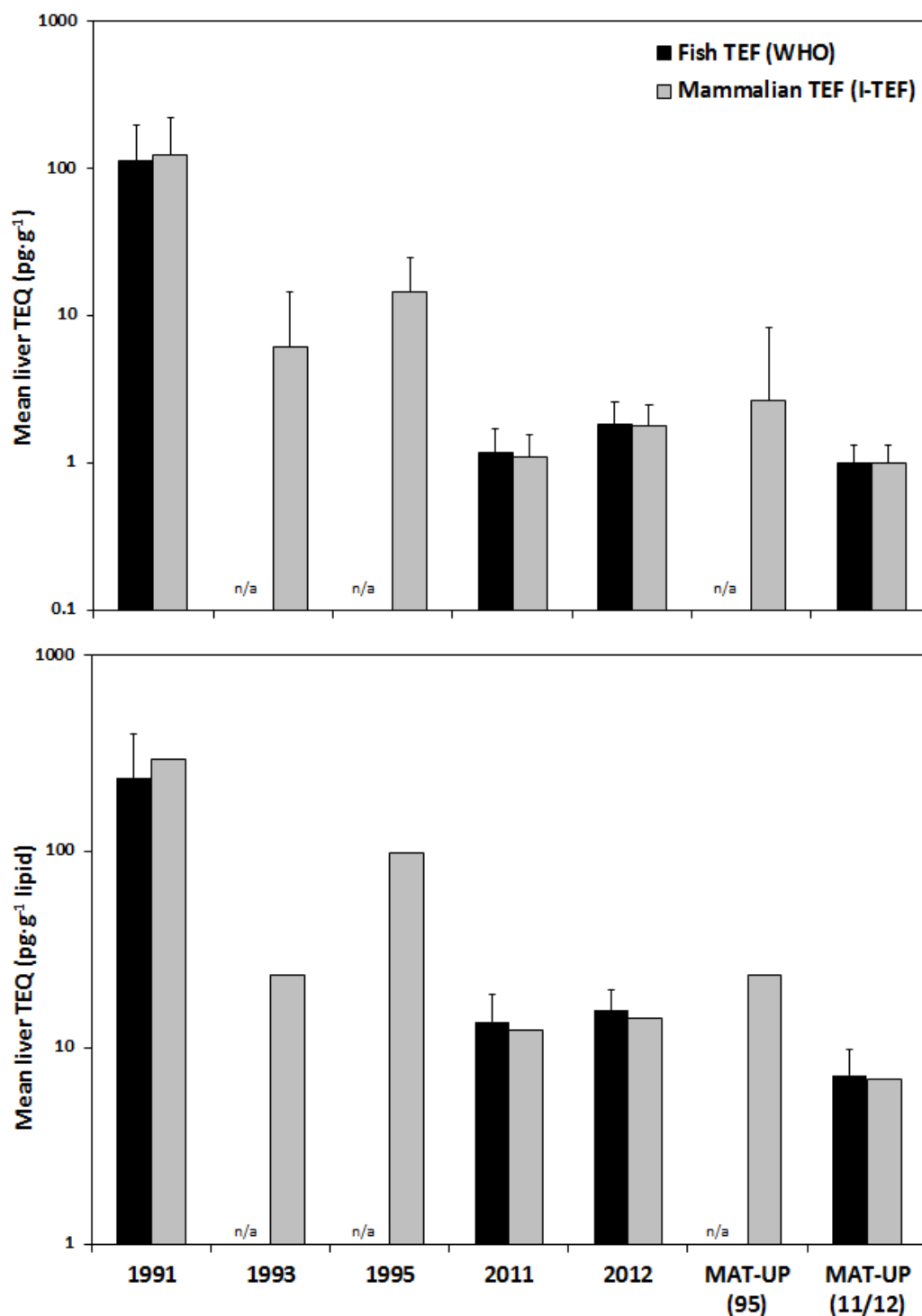


Figure 3.2 Mean liver TEQ (pg·g⁻¹ wet wt.) (top) and lipid normalized TEQ (pg·g⁻¹ lipid) (bottom) for liver tissue of male white sucker collected upstream (MAT-UP) and downstream of the pulp mill at Smooth Rock Falls, calculated using fish (black, Van den Berg et al., 1998) and mammalian (grey, Kutz et al., 1990) TEF. Bars represent mean TEQ ± SD and different letters indicate significant difference ($p < 0.05$, Student's t-test).

The contaminant profile observed in white sucker collected downstream of the mill in Smooth Rock Falls was dominated by 2,3,7,8-TCDD; 2,3,7,8-TCDF; 2,3,4,7,8-PeCDF; and 1,2,3,7,8-PeCDD in 1991. TEQ values were strongly influenced by 2,3,7,8-TCDD and 2,3,7,8-TCDF which represented 75% and 14%, respectively, of total TEQ calculated for exposed fish. The PCDD/F congener composition changed dramatically by 2011–2012 with the 2,3,7,8-TCDD and 2,3,7,8-TCDF becoming less influential to TEQ calculations and 1,2,3,4,7,8-HxCDD and 2,3,4,7,8-PeCDD becoming more important over time to represent a profile similar to that observed in fish collected upstream of the dam at Smooth Rock Falls.

3.5 Discussion

In 1991 a study investigating the response of the receiving environment to pulping process, effluent treatment and bleaching technology was conducted at several mills across northern Ontario (Munkittrick *et al.* 1994, Robinson *et al.* 1994, Servos *et al.* 1994, van den Heuvel *et al.* 1994). At the time of the 1991 survey the bleached kraft mill in Smooth Rock Falls employed Cl₂ as the bleaching agent with primary effluent treatment (Table 1.5, Chapter 1). White sucker collected downstream of the mill outfall demonstrated the most extensive biological response to effluent exposure, including MFO induction > 50 km downstream of Smooth Rock Falls (Munkittrick *et al.* 1994). Within one year of the installation of > 50% ClO₂ substitution a significant decrease in PCDD/F concentrations were observed in liver tissue of white sucker exposed to BKME in the Mattagami River (Servos *et al.* 1997). Similar trends have been observed in the receiving environment of other bleached kraft mills in Canada (Servos *et al.* 1997, Cater Chapter 2). Improvements in biological effects have been observed in the receiving environment of several bleached kraft pulp mills in North America (Munkittrick *et al.* 1997,

2013) and Sweden (Sandstrom and Neuman 2003) following implementation of ECF bleaching processes and upgraded effluent treatment. Complete recovery, however, has not been observed despite implementation of 100% ClO₂ substitution (McMaster *et al.* 2006, Munkittrick *et al.* 1992b, Munkittrick *et al.* 1997) and identification of the responsible compounds remains an area of active research (Hewitt *et al.* 2006, Kovacs *et al.* 2011).

High PCDD/F contamination measured in white sucker exposed to BKME from the pulp mill in Smooth Rock Falls in 1991 (111 ± 86.2 pg TEQ·g⁻¹) caused concern for ecosystem health and potential human health concerns; however, a dramatic decline in TEQ (wet wt. and lipid normalized) was observed in exposed fish following implementation of 100% ClO₂ in the bleaching process (Table 3.1, Fig. 3.1, 3.2). Servos *et al.* (1997) reported a rapid decline in TEQ measured in white sucker collected downstream of the outfall in 1993 compared to 1991. Mean TEQ of white sucker collected downstream between 1993 and 1995 appear to be approaching levels observed in pooled reference fish collected during fall 2011 and 2012. No difference was found in mean TEQ of fish collected downstream of the historical mill outfall in 2011 and 2012 compared to reference fish (1.00 ± 0.31 pg·g⁻¹) collected upstream during the same time period (Fig. 3.1). A similar decline was observed in lipid content of fish downstream of the mill with mean % lipid decreasing from 1991 ($46.8 \pm 10.7\%$) to 2012 ($12.5 \pm 5.56\%$). This decline corresponds to the introduction of 16% ClO₂ in the bleaching sequence in 1991 and subsequent upgrade to an ECF bleaching process through the use of 100% ClO₂ substitution (Table 1.3, Chapter 1). Secondary treatment was not installed at the pulp mill in Smooth Rock Falls until 1994 (Table 1.3, Chapter 1) and does not appear to have made a significant contribution to the removal of PCDD/Fs. These results are consistent with the observed trends in PCDD/F

contamination from the bleached kraft pulp mill in Terrace Bay, Ontario which discharges into Jackfish Bay, Lake Superior (Servos *et al.* 1997, Cater Chapter 2).

The return to background condition of TEQ values in white sucker collected downstream of the historical mill location follows the 2006 closure of the mill. The contaminant profile detected in white sucker exposed to BKME in the Mattagami River is consistent with the “bleaching sequence” observed in biota collected in the receiving environment of several bleached kraft mills (Bhavsar *et al.* 2008, Culp *et al.* 2000, De Vault *et al.* 1989, Huestis *et al.* 1997, Muir and Fraikin 2004, Whittle *et al.* 1993). In recent years, 2,3,7,8-TCDD and 2,3,7,8-TCDF concentrations in white sucker have declined dramatically and the congener sequence observed was similar to that of reference fish collected upstream of the dam. Further, the observed increase in the contribution of hepta (HpCDD/F) congeners to total TEQ calculations is indicative of the incineration (i.e., atmospheric) pattern of PCDD/Fs (Rappe *et al.* 1991, Swanson *et al.* 1988).

Because PCDD/Fs are highly lipophilic and tend to persist in sediment, they were expected to remain bioavailable in surface sediments and particulate (POM) for many years following their removal from effluents (Segstro *et al.* 1995). PCDD/F contamination has been detected in benthic invertebrates and bottom feeding fish for far distances downstream of the effluent discharge of many bleached kraft pulp mills (Whittle *et al.* 1993). The rapid decline in TEQ observed in bottom feeding white sucker in this study suggest that sediment did not provide a significant source of PCDD/F contamination to fish downstream of the Smooth Rock Falls mill following its removal from effluents. This is consistent with the nature of the Mattagami River which is a large, fast flowing system with few or no large sediment depositional areas immediately downstream of the mill. Despite considerable effort we were not able to collect

sediment cores for PCDD/F analysis below the dam in Smooth Rock Falls downstream as far as Little Long dam.

The Northern Rivers Basins Study (NRBS) contaminant program observed a similar pattern of declining PCDD/F contamination in fish following increased ClO₂ substitution at two bleached kraft pulp mills in northern Alberta (Muir and Fraikin 2004). The study also observed a decline in PCDD/F contamination of suspended solids (i.e., POM), uptake of which has been linked to accumulation of highly chlorinated PCDD/Fs in aquatic biota (Foster *et al.* 1999, Muir *et al.* 1992, Owens *et al.* 1994). It is possible that suspended solids are quickly swept downstream in large river systems, therefore eliminating the source of PCDD/Fs, allowing biota in the receiving environments to recover rapidly. These results support the evidence provided by a similar study conducted at Jackfish Bay, Lake Superior, which indicated that PCDD/F contamination of POM and DOM from mill effluent historically provided the primary route of uptake by fish in the BKME receiving environment (Cater Chapter 2). The rapid recovery observed in the Mattagami River, compared to Jackfish Bay, can likely be attributed to the closure of the mill as well as the different dynamics of a river system compared to a lake.

This study documented the temporal trends of PCDD/F contamination in the Mattagami River. A rapid decline in PCDD/F concentration was observed in liver tissue of bottom feeding white sucker following mill process upgrades to ECF bleaching practices. Following the 2006 closure of the pulp mill in Smooth Rock Falls, PCDD/F concentrations in white sucker exhibited a return to background levels. Although minor differences remain in lipid normalized TEQ values between reference and “exposed” fish collected in 2011 and 2012, these differences are likely not ecologically significant and ecosystem recovery is expected to continue with PCDD/F contamination remaining within observed background levels.

Chapter 4 General Conclusions

The research presented in this study provides insight into the legacy of polychlorinated dibenzo-*p*-dioxin (PCDD) and dibenzofuran (PCDF) contamination in bleached kraft pulp mill effluent (BKME) receiving environments. This was accomplished by examining PCDD/F concentrations in white sucker (*Catostomus commersoni*) and sediment following two decades of process changes and closures at the bleached kraft pulp mills in Terrace Bay and Smooth Rock Falls, Ontario. It was suspected that toxic equivalents (TEQ) measured in white sucker would reflect the legacy of PCDD/F contamination in surface sediment from each location.

High PCDD/F concentrations were measured in surface sediment throughout Jackfish Bay. In Moberly Bay (part of Jackfish Bay) concentrations were particularly high and exceeded the CCME probable effects level (PEL) established to indicate potential for adverse ecosystem effects. Despite elevated surface sediment concentrations, PCDD/Fs measured in fish illustrated a rapid decline following process upgrades to > 70% chlorine dioxide (ClO₂) substitution in the bleaching sequence. It is assumed that the freely dissolved concentrations of PCDD/Fs are extremely low and not important. This suggests that particulate (POM) and dissolved organic matter (DOM) from historical mill effluents played an important role in PCDD/F uptake by fish. Although surface sediment may contribute to uptake of PCDD/Fs, their bioavailability appears to decrease quickly once POM settles to the bottom of the water column and is buried. Ingestion of suspended solids and surface sediments by benthic invertebrates are integral to the transfer of lipophilic compounds in aquatic food chains. PCDD/F concentrations were not measured in benthic invertebrates from Jackfish Bay in the current study. Despite the recognition of Jackfish Bay as an area in recovery since 2010, the benthic community structure remains impaired and it

is unknown whether feeding dynamics have changed over the period of mill operation and how this may affect PCDD/F contamination of white sucker.

Analysis of dated sediment cores from Moberly Bay illustrated a link between process upgrades at the mill and changes in PCDD/F concentrations and congener patterns in buried sediment. PCDD/F contamination of fish and sediment was consistently dominated by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and dibenzofuran (TCDF). The high concentrations of 2,3,7,8-TCDF observed in historically contaminated sediment was attributed to the historical use of oil-based defoamers and brown stock washers containing furan precursors. The observed congener sequence is consistent with the “bleaching pattern” observed in sediment and biota exposed to BKME worldwide. This pattern was also evident in white sucker collected in the Mattagami River downstream of the Smooth Rock Falls pulp mill in 1991 when the mill employed primary effluent treatment and molecular chlorine (Cl₂) bleaching processes. Despite considerable effort, suitable sediment depositional areas were not found for the collection of sediment cores in the Mattagami River. Because the Mattagami River is a large, fast flowing river it is likely that PCDD/F contaminated POM (suspended and surface sediments) and DOM were quickly swept downstream and removed from the system once continuous effluent discharges from the mill were eliminated.

This study has contributed to further understanding the recovery of ecosystems from pulp and paper mill effluents. In the Mattagami River, PCDD/F concentrations in white sucker were dramatically reduced following mill modernization and have returned to reference condition since its closure in 2006. It is likely that with no effluent discharging into the Mattagami River, the ecosystem downstream of the historical mill outfall will continue to recover and PCDD/F contamination will remain near background levels. At Jackfish Bay, PCDD/F concentrations

measured in liver tissue of white sucker collected during 2011 and 2012 were approaching background levels observed in reference fish from Mountain Bay; however, a complete return to reference condition may take a long time as the sediments become removed or buried. The bleached kraft pulp mill in Terrace Bay continues to discharge effluent into Jackfish Bay and it is expected to be converted to a dissolving process over the next 2–3 years. PCDD/F inputs to Jackfish Bay are expected to continue to be very low and PCDD/F contamination of white sucker will continue to gradually decline toward background levels.

Levels of PCDD/Fs in the aquatic environment of both Jackfish Bay and the Mattagami River have declined dramatically with the implementation of treatment and process changes in the early 1990s. Although elevated levels of PCDD/Fs may remain, especially in depositional areas, they do not appear to be readily bioavailable. The contamination of white sucker are approaching or similar to PCDD/F concentrations observed at reference sites and are below the levels of concern for ecosystem health or human consumption. The actions taken by the pulp and paper industry to address the environmental contamination from PCDD/Fs is clearly an international success story of environmental stewardship and regulatory success.

References

ACRES (1996) Environmental Effect Monitoring for Mallette Kraft Pulp & Power: First cycle report. Acres International Limited, Toronto, ON, Canada.

Aditya Birla Management Corporation Pvt. Ltd. (2012). Terrace Bay pulp mill, from <http://www.adityabirla.com/about/terrace-bay-pulp-mill>

Allen, L.H., Berry, R.M., Fleming, B.I., Luthé, C.E. and Voss, R.H. (1989) Evidence that oil-based additives are an indirect source of the TCDD and TCDF produced in kraft bleach plants. *Chemosphere* 19(1–6), 741-744.

Amendola, G., Barna, D., Blosser, R., LaFleur, L., McBride, A., Thomas, F., Tiernan, T. and Whittemore, R. (1989) The occurrence and fate of PCDDs and PCDFs in five bleached kraft pulp and paper mills. *Chemosphere* 18(1–6), 1181-1188.

Andersson, T. and Förlin, L. (1992) Regulation of the cytochrome P450 enzyme system in fish. *Aquatic Toxicology* 24(1), 1-19.

Appleby, P.G. (2001) Chronostratigraphic techniques in recent sediments. *In*: W.M. Last and J.P. Smol (Eds.), *Tracking environmental change using lake sediments: Basin analysis, coring, and chronological techniques*, pp. 171-203, Dordrecht, NL: Kluwer Academic Publishers

Arciszewski, T.J., Farwell, A., Servos, M.R., Munkittrick, K.R. (2013) Stable carbon and nitrogen in tissues of white sucker (*Catostomus commersoni*) before and after the closure of a bleached kraft mill. Submitted.

Axegård, P. and Renberg, L. (1989) The influence of bleaching chemicals and lignin content on the formation of polychlorinated dioxins and dibenzofurans. *Chemosphere* 19(1), 661-668.

AXYS Analytical Services Ltd. (2012). AXYS Method MLA-017: Analytical method for the determination of polychlorinated dibenzodioxins and dibenzofurans (Rev 20 Ver 04).

Bajpai, P. (2012) *Biotechnology for pulp and paper processing*, New York, USA: Springer Science & Business Media.

Bajpai, P. (2013). Bleach Plant Effluents from the Pulp and Paper Industry Pulp Bleaching and Bleaching Effluents, pp. 13-19, New York, USA: Springer International Publishing.

BEAK (1995) Kimberly-Clark Forest Products Inc. Terrace Bay. Pre-design and study design. BEAK Consulting Limited, Brampton, ON, Canada.

BEAK (1996) First cycle Environmental Effects Monitoring study - 1995. Draft report prepared for Kimberly-Clark Forest Products Inc. Terrace Bay, Ontario. BEAK Consultants Limited, Brampton, ON, Canada.

BEAK (1998) EEM Cycle 2 Pre-design and study design for Kimberly-Clark Forest Products Inc., Terrace Bay. BEAK International Incorporated, Brampton, ON, Canada.

BEAK (2000) Second Cycle EEM final interpretive report for Kimberly-Clark Incorporated. BEAK Consulting Limited., Brampton, ON, Canada.

Berry, R., Fleming, B., Voss, R., Luthe, C. and Wrist, P. (1989) Toward preventing the formation of dioxins during chemical pulp bleaching. *Pulp & Paper Canada* 90(8), 48-58.

Berry, R., Luthe, C., Voss, R. and Wrist, P. (1991) The effects of recent changes in bleached softwood kraft mill technology on organochlorine emissions: An international perspective. *Pulp & Paper Canada* 92(6).

Bhavsar, S.P., Awad, E., Fletcher, R., Hayton, A., Somers, K.M., Kolic, T., MacPherson, K. and Reiner, E.J. (2008) Temporal trends and spatial distribution of dioxins and furans in lake trout or lake whitefish from the Canadian Great Lakes. *Chemosphere* 73(1, Supplement), S158-S165.

Binford, M.W. (1990) Calculation and uncertainty analysis of ²¹⁰Pb dates for PIRLA project lake sediment cores. *Journal of Paleolimnology* 3(3), 253-267.

Bowron, L.K., Munkittrick, K.R., McMaster, M.E., Tetreault, G. and Hewitt, L.M. (2009) Responses of white sucker (*Catostomus commersoni*) to 20 years of process and waste treatment changes at a bleached kraft pulp mill, and to mill shutdown. *Aquatic Toxicology* 95(2), 117-132.

Brousseau, C.S., and Goodchild, G.A. (1989). Fisheries and yields in the Moose River Basin, Ontario. In D.P.Dodge. (Ed.), *Proceedings of the International Large River Symposium*, Honey Harbour, Ontario, Canada 1986. Can.Spec. Publ. Fish. Aquat. Sci. No. 106. pp. 145–158.

Cater, S.C (2013) Chapter 2: Historical trends of polychlorinated dibenzo-p-dioxins and dibenzofurans in white sucker (*Catostomus commersoni*) liver and dated sediment cores from Jackfish Bay, Lake Superior. MSc Thesis. University of Waterloo, Waterloo, ON, Canada.

CCME (Canadian Council of Ministers of the Environment) (2001a) Canadian Environmental Quality Guidelines. Canadian Sediment Quality Guidelines for the Protection of Aquatic Life. Updated. In: Canadian Environmental Quality Guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg, MN, Canada.

CCME (2001b) Canadian tissue residue guidelines for the protection of wildlife consumers of aquatic biota: Introduction. Updated. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg, MN, Canada.

CEPA (Canadian Environmental Protection Act) (1999) Pulp and Paper Mill Effluent Chlorinated Dioxins and, Furans Regulations (SOR/92-267), Ottawa, ON, Canada.

Clemons, J.H., Dixon, D.G. and Bols, N.C. (1997) Derivation of 2,3,7,8-TCDD toxic equivalence factors (TEFs) for selected dioxins, furans and PCBs with rainbow trout and rat liver cell lines and the influence of exposure time. *Chemosphere* 34(5-7), 1105-1119.

Comba, M.E., Palabrica, V.S. and Kaiser, K.L.E. (1994) Volatile halocarbons as tracers of pulp mill effluent plumes. *Environmental Toxicology and Chemistry* 13(7), 1065-1074.

Culp, J.M., Cash, K.J. and Wrona, F.J. (2000) Cumulative effects assessment for the northern river basins study. *Journal of Aquatic Ecosystem Stress and Recovery* 8(1), 87-94.

Currie, L.A. (1968) Limits for qualitative detection and quantitative determination. Application to radiochemistry. *Analytical Chemistry* 40(3), 586-593.

De Vault, D., Dunn, W., Bergqvist, P.A., Wiberg, K. and Rappe, C. (1989) Polychlorinated dibenzofurans and polychlorinated dibenzo-p-dioxins in Great Lakes fish: A baseline and interlake comparison. *Environmental Toxicology and Chemistry* 8(11), 1013-1022.

Delescluse, C., Lemaire, G., de Sousa, G. and Rahmani, R. (2000) Is CYP1A1 induction always related to AHR signaling pathway? *Toxicology* 153(1-3), 73-82.

Dougherty, R.C. Human exposure to pentachlorophenol. Pentachlorophenol chemistry, pharmacology, and environmental toxicology, *Environmental Science Research* 12, ed. K. R. Rao, Plenum Press: New York, p. 355, 1978.

Elliott, J., Whitehead, P., Martin, P., Bellward, G., and Norstrom, R. (1996). Persistent pulp mill pollutants in wildlife. *In* M. R. Servos, K. R. Munkittrick, J. H. Carey and G. J. Van Der Kraak (Eds.), *Environmental Fate and Effects of Pulp and Paper Mill Effluents*, pp. 297-314, Delray Beach, FL, USA: St. Lucie Press.

Environment Canada (1998) Pulp and paper technical guidance for environmental effects monitoring (EEM/1998/8), Environment Canada, Ottawa, ON, Canada.

Environment Canada & OMOE (Ontario Ministry of the Environment) (2011). *Jackfish Bay Area in Recovery* status of beneficial use impairments. ISBN: 978-1-100-18066-3.

ESG (1998) Environmental effects monitoring cycle II pre-design and study design Malette Kraft Pulp and Power Co. ESG International, Guelph, ON, Canada.

ESG (2000) Environmental Effects Monitoring (EEM) cycle II final report Tembec Inc. Smooth Rock Falls division. ESG International., Guelph, ON, Canada.

Fletcher, C.L. and McKay, W.A. (1993) Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in the aquatic environment — a literature review. *Chemosphere* 26(6), 1041-1069.

Foster, E.P., Drake, D. and Farlow, R. (1999) Polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran congener profiles in fish, crayfish, and sediment collected near a wood treating facility and a bleached kraft pulp mill. *Bulletin of Environmental Contamination and Toxicology* 62(3), 239-246.

Friesen, K.J., Vilks, J. and Muir, D.C.G. (1990) Aqueous solubilities of selected 2,3,7,8-substituted polychlorinated dibenzofurans (PCDFs). *Chemosphere* 20(1-2), 27-32.

Geyer, H.J., Muir, D.C.G., Scheunert, I., Steinberg, C.E.W. and Kettrup, A.A.W. (1992) Bioconcentration of octachlorodibenzo-*p*-dioxin (OCDD) in fish. *Chemosphere* 25(7-10), 1257-1264.

Hahn, M.E. (2001) Dioxin toxicology and the aryl hydrocarbon receptor: insights from fish and other non-traditional models. *Marine Biotechnology* 3, S224-S238.

Hebert, C.E. and Keenleyside, K.A. (1995) To normalize or not to normalize? Fat is the question. *Environmental Toxicology and Chemistry* 14(5), 801-807.

Hewitt, L.M., Parrott, J.L. and McMaster, M.E. (2006) A decade of research on the environmental impacts of pulp and paper mill effluents in Canada: Sources and characteristics of bioactive substances. *Journal of Toxicology and Environmental Health-Part B-Critical Reviews* 9(4), 341-356.

Hodgson, E. and Dauterman, W.C. (1980) Metabolism of toxicants: Phase I reactions. *In* Hodgson, E. and Guthrie, F.E. (eds), *Introduction to biochemical toxicology*, ISBN: 9780471333340, pp. 67-90, Elsevier, New York, USA.

Hodson, P.V., Thivierge, D., Levesque, M.C., McWhirter, M., Ralph, K., Gray, B., Whittle, D.M., Carey, J.H. and Van Der Kraak, G. (1992) Effects of bleached kraft mill effluent on fish in the St. Maurice River, Quebec. *Environmental Toxicology and Chemistry* 11(11), 1635-1651.

Huestis, S.Y., Servos, M.R., Whittle, D.M., vandenHeuvel, M. and Dixon, D.G. (1997) Evaluation of temporal and age-related trends of chemically and biologically generated 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents in Lake Ontario lake trout, 1977 to 1993. *Environmental Toxicology and Chemistry* 16(2), 154-164.

Jackfish Bay Remediation Action Plan (RAP) Team (1991). Stage 1: Environmental conditions and problem definitions, Jackfish Bay RAP, ISBN: 0-7729-9034-4, North Shore of Lake Superior Remedial Action Plans. Thunder Bay, ON, Canada.

Jackfish Bay RAP Team (1998). Stage 2: Remedial strategies for ecosystem restoration, Jackfish Bay RAP, North Shore of Lake Superior Remedial Action Plans. Thunder Bay, ON, Canada.

Jones, P.D., Kannan, K., Newsted, J.L., Tillitt, D.E., Williams, L.L. and Giesy, J.P. (2001) Accumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin by rainbow trout (*Onchorhynchus mykiss*) at environmentally relevant dietary concentrations. *Environmental Toxicology and Chemistry* 20(2), 344-350.

Kovacs, T.G., Martel, P.H., O'Connor, B.I., Parrott, J.L., McMaster, M.E., Van Der Kraak, G.J., MacLachy, D.L., van den Heuvel, M.R. and Hewitt, L.M. (2011) Kraft mill effluent survey: Progress toward best management practices for reducing effects on fish reproduction. *Environmental Toxicology and Chemistry* 30(6), 1421-1429.

Kuehl, D.W., Butterworth, B.C., Devita, W.M. and Sauer, C.P. (1987) Environmental contamination by polychlorinated dibenzo-p-dioxins and dibenzofurans associated with pulp and paper mill discharge. *Biological Mass Spectrometry* 14(8), 443-447.

Kutz, F.W., Barnes, D.G., Bottimore, D.P., Greim, H. and Bretthauer, E.W. (1990) The international toxicity equivalency factor (I-TEF) method of risk assessment for complex mixtures of dioxins and related compounds. *Chemosphere* 20(7-9), 751-757.

LaFleur, L., Brunck, B., McDonough, T., Ramage, K., Gillespie, W. and Malcolm, E. (1990) Studies on the mechanism of PCDD/PCDF formation during the bleaching of pulp. *Chemosphere* 20(10-12), 1731-1738.

Larisch, B. and Duff, S. (1997) Effect of H₂O₂ and DTPA on the characteristics and treatment of TCF (totally chlorine-free) and ECF (elementally chlorine-free) kraft pulping effluents. *Water Science and Technology* 35(2), 163-171.

Lindström-Seppä, P., Huuskonen, S., Pesonen, M., Muona, P. and Hanninen, O. (1992) Unbleached pulp mill effluents affect cytochrome-P450 monooxygenase enzyme activities. *Marine Environmental Research* (34), 157-161.

Liu, F. (2007) Microbial dechlorination of polychlorinated dibenzo-p-dioxins and dibenzofurans: pathways, kinetics and environmental implications. PhD Thesis. Rutgers University, New Jersey, USA.

Luthe, C.E., Wrist, P. and Berry, R. (1992) An evaluation of the effectiveness of dioxins control strategies on organochlorine effluent discharges from the Canadian bleached chemical pulp industry: four surveys show the effort and the achievements. *Pulp & Paper Canada* 93(9), 40-49.

Luthe, C.E. and Berry, R.M. (1996) The role of dibenzo-p-dioxin and dibenzofuran precursors in the formation of tetrachlorinated dibenzo-p-dioxins/-furans during bleaching. *Chemosphere* 32(5), 881-891.

Luthe, C.E. (1998) Progress in reducing dioxins and AOX: A Canadian perspective. *Chemosphere* 36(2), 225-229.

Mandal, P.K. (2005) Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 175(4), 221-230.

McDonough, T.J. (1995) Recent advances in bleached chemical pulp manufacturing technology 1. Extended delignification, oxygen delignification, enzyme applications, and ECF and TCF bleaching. *Tappi Journal* 78(3), 55-62.

McKee, P., Burt, A. and McCurvin, D. (1990) Levels of dioxins, furans and other organic contaminants in harbour sediments near a wood preserving plant using pentachlorophenol and creosote. *Chemosphere* 20 (10), 1679-1685.

McMaster, M.E., Vanderkraak, G.J., Portt, C.B., Munkittrick, K.R., Sibley, P.K., Smith, I.R. and Dixon, D.G. (1991) Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid-levels and age at maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft pulp mill effluent. *Aquatic Toxicology* 21(3-4), 199-218.

McMaster, M.E., Hewitt, L.M. and Parrott, J.L. (2006) A decade of research on the environmental impacts of pulp and paper mill effluents in Canada: Field studies and mechanistic research. *Journal of Toxicology and Environmental Health-Part B-Critical Reviews* 9(4), 319-339.

Milani, D. (2009) Benthic conditions in the Jackfish Bay Area of Concern 2008, Environment Canada, Burlington, ON, Science and Technology Directorate (WSTD No. 09-541).

Miller, M.A., Madenjian, C.P. and Masnado, R.G. (1992) Patterns of organochlorine contamination in lake trout from Wisconsin waters of the Great Lakes. *Journal of Great Lakes Research* 18(4), 742-754.

Muir, D. and Fraikin, C. (2004) Spatial and temporal trends of organochlorine contaminants in fish from Alberta's northern rivers. Environment Canada, Northern Rivers Ecosystem Initiative: Collective Findings (CDROM). Compiled by F.M., Conly, Saskatoon, SK, Canada.

Muir, D.C.G., Lawrence, S., Holoka, M., Fairchild, W.L., Segstro, M.D., Webster, G.R.B. and Servos, M.R. (1992) Partitioning of polychlorinated dioxins and furans between water, sediments and biota in lake mesocosms. *Chemosphere* 25(1-2), 119-124.

Muir, D.C.G. and Servos, M.R. (1996) Bioaccumulation of bleached kraft pulp mill related organic chemicals by fish. *In* M. R. Servos, K. R. Munkittrick, J. H. Carey and G. J. Van Der Kraak (Eds.), *Environmental Fate and Effects of Pulp and Paper Mill Effluents*, pp. 283-295, Delray Beach, FL, USA: St. Lucie Press.

Munkittrick, K., Van Der Kraak, G., McMaster, M. and Portt, C. (1992a) Reproductive dysfunction and MFO activity in three species of fish exposed to bleached kraft mill effluent at Jackfish Bay, Lake Superior. *Water Pollution Research Journal of Canada*.

Munkittrick, K.R., Portt, C.B., Van Der Kraak, G.J., Smith, I.R. and Rokosh, D.A. (1991) Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. Canadian Journal of Fisheries and Aquatic Sciences 48(8), 1371-1380.

Munkittrick, K.R., Van Der Kraak, G.J., McMaster, M.E. and Portt, C.B. (1992b) Response of hepatic MFO activity and plasma sex steroids to secondary treatment of bleached kraft pulp mill effluent and mill shutdown. Environmental Toxicology and Chemistry 11(10), 1427-1439.

Munkittrick, K.R., Van Der Kraak, G.J., McMaster, M.E., Portt, C.B., van den Heuvel, M.R. and Servos, M.R. (1994) Survey of receiving water environmental impacts associated with discharge from pulp mills 2. Gonad size, liver size hepatic EROD activity and plasma sex steroid levels in white sucker. Environmental Toxicology and Chemistry 13(7), 1089-1101.

Munkittrick, K.R., Servos, M.R., Carey, J.H. and Van Der Kraak, G.J. (1997) Environmental impacts of pulp and paper wastewater: Evidence for a reduction in environmental effects at North American pulp mills since 1992. Water Science and Technology 35(2-3), 329-338.

Munkittrick, K.R., McMaster, M.E., Van Der Kraak, G.J., Portt, C., Gibbons, W.N., Farwell, A. and Gray, M. (2000) Development of methods for effects-driven cumulative effects assessment using fish populations: Moose River project, Pensacola, FL, USA.

Munkittrick, K.R., McMaster, M.E. and Servos, M.R. (2013) Detection of reproductive impacts of effluents from pulp and paper mills: Shifts in issues and potential causes. Environmental Toxicology and Chemistry 32(4), 729-731.

Nebert, D.W. and Gonzalez, F. (1987) P450 genes: structure, evolution, and regulation. Annual Review of Biochemistry 56(1), 945-993.

Nickle, J., McMaster, M., Munkittrick, K., Rumsey, C., Portt, C. and Van Der Kraak, G. (1997) Reproductive effects of primary treated bleached kraft and chemi-thermomechanical pulp mill effluent on white sucker (*Catostomus commersoni*) in the Moose River basin. In Stuthridge, T.R., van den Heuvel, M.R., Marvin, N., Slade, A. and Gifford, J. (eds) Environmental Impacts of Pulp and Paper Waste Streams. Proceedings of the 3rd International Conference on the Fate and effects of Pulp and Paper Mill Effluents, Rotorua, New Zealand, 1997, pp. 208-215, SETAC Press, Pensacola, FL, USA.

Oinonen, T. and Lindros, K. (1998) Zonation of hepatic cytochrome P-450 expression and regulation. Biochemical Journal 329, 17-35.

Owens, J., Swanson, S. and Birkholz, D. (1994) Bioaccumulation of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, 2, 3, 7, 8-tetrachlorodibenzofuran and extractable organic chlorine at a bleached-kraft mill site in a northern Canadian river system. *Environmental Toxicology and Chemistry* 13(2), 343-354.

Parrott, J.L., Dixon, D.G., Hodson, P.V., Servos, M.R. and Huestis, U.L. (1995) Relative potency of polychlorinated dibenzo-p-dioxins and dibenzofurans for inducing mixed-function oxygenase activity in rainbow trout. *Environmental Toxicology and Chemistry* 14(6), 1041-1050.

Parthasarthy, V., Rudie, G., Mendiratta, S., Cawifield, D. and Bryan, H. (1994) Gaseous chlorine dioxide delignification of softwood pulps for high brightness and ultra low AOX. *In: Proceedings of Tappi Pulping Conference, San Diego, USA, 1994, Tappi Journal, San Diego, CA, USA*

Pearson, R.F., Swackhamer, D.L., Eisenreich, S.J. and Long, D.T. (1998) Atmospheric Inputs of Polychlorinated dibenzo-p-dioxins and dibenzofurans to the Great Lakes: Compositional comparison of PCDD and PCDF in sediments. *Journal of Great Lakes Research* 24(1), 65-82.

Poland, A. and Knutson, J.C. (1982) 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annual Reviews of Pharmacology and Toxicology* 22, 517-554.

Rappe, C., Andersson, R., Bergqvist, P.-A., Brohede, C., Hansson, M., Kjeller, L.O., Lindström, G., Marklund, S., Nygren, M. and Swanson, S.E. (1987) Overview on environmental fate of chlorinated dioxins and dibenzofurans: Sources, levels and isomeric pattern in various matrices. *Chemosphere* 16(8), 1603-1618.

Rappe, C., Swanson, S.E., Glas, B., Kringstad, K.P., Desousa, P. and Abe, Z. (1989) Formation of PCDDs and PCDFs by the chlorination of water. *Chemosphere* 19(12), 1875-1880.

Rappe, C., Kjeller, L.O., Kulp, S.E., Dewit, C., Hasselsten, I. and Palm, O. (1991) Levels, profile and pattern of PCDDs and PCDFs in samples related to the production and use of chlorine. *Chemosphere* 23(11-12), 1629-1636.

Robinson, R.D., Carey, J.H., Solomon, K.R., Smith, I.R., Servos, M.R. and Munkittrick, K.R. (1994) Survey of receiving water environmental impacts associated with discharges from pulp mills 1. Mill characteristics, receiving water chemical profiles and lab toxicity tests. *Environmental Toxicology and Chemistry* 13(7), 1075-1088.

Sandstrom, O., Neuman, E. and Karas, P. (1988) Effects of a bleached pulp mill effluent on growth and gonad function in Baltic coastal fish. *Water Science and Technology* 20(2), 107-118.

Sandstrom, O. and Neuman, E. (2003) Long-term development in a Baltic fish community exposed to bleached pulp mill effluent. *Aquatic Ecology* 37(3), 267-276.

Segstro, M.D., Muir, D.C.G., Servos, M.R. and Webster, G.R.B. (1995) Long-term fate and bioavailability of sediment-associated polychlorinated dibenzo-p-dioxins in aquatic mesocosms. *Environmental Toxicology and Chemistry* 14(10), 1799-1807.

Servos, M.R., Muir, D.C.G. and Webster, G.R.B. (1989a) The effect of dissolved organic matter on the bioavailability of polychlorinated dibenzo-para-dioxins. *Aquatic Toxicology* 14(2), 169-184.

Servos, M.R., Muir, D.C.G., Whittle, D.M., Sergeant, D.B. and Webster, G.R.B. (1989b) Bioavailability of octachlorodibenzo-p-dioxin in aquatic ecosystems. *Chemosphere* 19(1-6), 969-972.

Servos, M.R., Carey, J.H., Ferguson, M.L., Van Der Kraak, G.J., Ferguson, H., Parrott, J.L., Gorman, K. and Cowling, R. (1992a) Impact of a modernized bleached kraft mill on white sucker populations in the Spanish River, Ontario. *Water Pollution Research Journal of Canada* 27(3), 423-437.

Servos, M.R., Muir, D.C.G. and Webster, G.R.B. (1992b) Environmental fate of polychlorinated dibenzo-p-dioxins in lake enclosures. *Canadian Journal of Fisheries and Aquatic Sciences* 49(4), 722-734.

Servos, M.R., Muir, D.C.G. and Webster, G.R.B. (1992c) Bioavailability of polychlorinated dibenzo-p-dioxins in lake enclosures. *Canadian Journal of Fisheries and Aquatic Sciences* 49(4), 735-742.

Servos, M.R., Huestis, S.Y., Whittle, M.D., Munkittrick, K.R. and Van Der Kraak, G.J. (1994) Survey of receiving-water environmental impacts associated with discharges from pulp mills: 3. Polychlorinated dioxins and furans in muscle and liver of white sucker (*Catostomus commersoni*). *Environmental Toxicology and Chemistry* 13(7), 1103-1115.

Servos, M.R., Luce, S., Toito, J., McMaster, M.E., Munkittrick, K.R., Huestis, S.Y., Hagen, M.E. and Colodey, A.G. (1997) In Stuthridge, T.R., van den Heuvel, M.R., Marvin, N., Slade, A. and Gifford, J. (eds) *Environmental Impacts of Pulp and Paper Waste Streams*. Proceedings of the 3rd International Conference on the Fate and effects of Pulp and Paper Mill Effluents, Rotorua, New Zealand, 1997., pp. 208-215, SETAC Press, Pensacola, FL, USA.

Sherman, R.K., Clement, R.E. and Tashiro, C. (1990) The distribution of polychlorinated dibenzo-para-dioxins and dibenzofurans in Jackfish Bay, Lake Superior, in relation to a kraft pulp-mill effluent. *Chemosphere* 20(10-12), 1641-1648.

Shiu, W.Y., Doucette, W., Gobas, F.A.P.C., Andren, A. and Mackay, D. (1988) Physical-chemical properties of chlorinated dibenzo-p-dioxins. *Environmental Science & Technology* 22(6), 651-658.

Sibley, P.K., Legler, J., Dixon, D.G. and Barton, D.R. (1997) Environmental health assessment of the benthic habitat adjacent to a pulp mill discharge 1. Acute and chronic toxicity of sediments to benthic macroinvertebrates. *Archives of Environmental Contamination and Toxicology* 32(3), 274-284.

Sibley, P.K., Dixon, D.G. and Barton, D.R. (1998) Environmental assessment of benthic impacts associated with pulp mill discharges. II. Distribution of sediment EOX in relation to environmental factors. *Archives of Environmental Contamination and Toxicology* 34(2), 158-166.

Sibley, P.K., Dixon, D.G. and Barton, D.R. (2000) Impact of bleached kraft pulp mill effluent on benthic community structure in relation to environmental factors. *Journal of Aquatic Ecosystem Stress and Recovery* 7(3), 229-246.

Sibley, P.K., Dixon, D.G. and Barton, D.R. (2001) Impact of bleached kraft pulp mill effluent on the nearshore benthic community of Jackfish Bay, Lake Superior. *Water Quality Research Journal of Canada* 36(4), 815-833.

Smook, G.A. (1989) Overview of the pulp and paper industry from a chemical industry perspective. *Journal of Chemical Technology and Biotechnology* 45(1), 15-27.

Smook, G.A. (1992) *Handbook for Pulp and Paper Technologists*, 2nd ed. Angus Wide Publications, Vancouver, BC, Canada.

Södergren, A. (1989) Biological effects of bleached pulp mill effluents. Final Report from the Environment/Cellulose 1 Project. National Swedish Environmental Protection Board. Bratts Tryckeri AB, Jököping, Sweden.

Stantec (2004a) Cycle 3 Environmental Effects Monitoring Tembec Inc., Smooth Rock Falls division final report. Stantec Consulting Ltd., Guelph, ON, Canada.

Stantec (2004b) Cycle 3 Environmental Effects Monitoring Report for the Kimberly-Clark Inc., Terrace Bay mill. Stantec Consulting Inc., Brampton, ON, Canada.

Stantec (2005) Tembec Inc., Smooth Rock Falls Division Cycle 4 EEM pre-design and study design report. Stantec Consulting Inc., Guelph, ON, Canada.

Stantec (2007) Cycle 4 Environmental Effects Monitoring final report prepared for Tembec Industries Inc. Smooth Rock Falls Division. Stantec Consulting Ltd., Guelph, ON, Canada.

Stewart, R.M. and Rashid, H. (2011) Blending science and public policies for remediation of a degraded ecosystem: Jackfish Bay, north shore of Lake Superior, Ontario, Canada. *Journal of Great Lakes Research* 37(2), 256-262.

Stow, C.A., Jackson, L.J. and Amrhein, J.F. (1997) An examination of the PCB:lipid relationship among individual fish. *Canadian Journal of Fisheries and Aquatic Sciences* 54(5), 1031-1038.

Swanson, S.E., Rappe, C., Malmstrom, J. and Kringstad, K.P. (1988) Emissions of PCDDs and PCDFs from the pulp industry. *Chemosphere* 17(4), 681-691.

Systat Software (2012). SigmaPlot 12.3. San Jose, CA, USA.

Thomann, R.V. (1989) Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environmental Science & Technology* 23(6), 699-707.

US-EPA (1993) Interim report on data and methods for assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin risks to aquatic life and associated wildlife. EPA/600/R-93/055.

Cook, P.M., Erickson, R.J., Spehar, R.L., Bradbury, S.P and Ankley, G.T. (Eds.) Office of Research and Development, US Environmental Protection Agency, Duluth, MN, USA.

US-EPA. (1994). Method 8290, Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). September 1994. US Environmental Protection Agency.

US-EPA. (1994). Method 1613, Tetra- through octa- chlorinated dioxins and furans by isotope dilution HRGC/HRMS. Revision B, October 1994. US Environmental Protection Agency, Office of Water, Washington, DC, USA.

US-EPA. (1998). Method 8290A, Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). Revision 1, January 1998. US Environmental Protection Agency.

Van den Berg, M., Birnbaum, L., Bosveld, A., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R. and Kennedy, S.W. (1998) Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental Health Perspectives* 106(12), 775.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N. and Peterson, R.E. (2006) The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences* 93(2), 223-241.

van den Heuvel, M.R., Munkittrick, K.R., Van Der Kraak, G.J., McMaster, M.E., Portt, C.B., Servos, M.R. and Dixon, D.G. (1994) Survey of receiving water environmental impacts associated with discharges from pulp mills 4. Bioassay derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalent concentration in white sucker (*Catostomus commersoni*) in relation to biochemical indicators of impact. *Environmental Toxicology and Chemistry* 13(7), 1117-1126.

Voiland, M.P., Gall, K.L., Lisk, D.J. and MacNeill, D.B. (1991) Effectiveness of recommended fat-trimming procedures on the reduction of PCB and Mirex levels in brown trout (*Salmo trutta*) from Lake Ontario. *Journal of Great Lakes Research* 17(4), 454-460.

Voss, R., Luthe, C., Fleming, B., Berry, R. and Allen, L. (1988) Some new insights into the origins of dioxins formed during chemical pulp bleaching. *Pulp and Paper Canada* 89(12), 151-162.

Walker, C.H., Hopkin, S.P., Sibly, R.M. and Peakall, D.B (2006) *Principles of Ecotoxicology* . 3rd Ed., Boca Raton, FL: CRC Press, Taylor & Francis Group.

Walker, S.L., Hedley, K. and Porter, E. (2002) Pulp and paper environmental effects monitoring in Canada: An overview. *Water Quality Research Journal of Canada* 37(1), 7-19.

Whitlock, J.P., Okino, S.T., Dong, L.Q., Ko, H.S.P., ClarkeKatzenberg, R., Qiang, M. and Li, H. (1996) Cytochromes P450 5: Induction of cytochrome P4501A1: A model for analyzing mammalian gene transcription. *Faseb Journal* 10(8), 809-818.

Whittle, D.M., Sergeant, D.B., Huestis, S.Y. and Hyatt, W.H. (1992) Foodchain accumulation of PCDD and PCDF isomers in the Great Lakes aquatic community. *Chemosphere* 25(7-10), 1559-1563.

Whittle, D.M., Mageau, C., Duncan, R.K., Sergeant, D.B., Nassichuk, M.D., Morrisson, J. and Piuze, J. (1993) Canadian national dioxin sampling program: dioxins and furans in biota near 46 pulp and paper mills using the chlorine bleaching process. *Chemosphere* 27(1-3), 279-286.

Wiseman, S. (2007) Characterization of AhR Signaling and the Impact of Polychlorinated Biphenyls on the Adaptive Responses to Stress in Fish. PhD Thesis. University of Waterloo, Waterloo, ON, Canada.

Yunker, M.B. and Cretney, W.J. (1996) Dioxins and furans in crab hepatopancreas: Use of principal components analysis to classify congener patterns and determine linkages to contamination sources. *In* Servos, M. R., Munkittrick, K. R., Carey, J. H. and Van Der Kraak, G.J. (Eds.), *Environmental Fate and Effects of Pulp and Paper Mill Effluents*, pp. 315-325, Delray Beach, FL, USA: St. Lucie Press.

Yunker, M.B., Cretney, W.J. and Ikonomou, M.G. (2002) Assessment of chlorinated dibenzo-p-dioxin and dibenzofuran trends in sediment and crab hepatopancreas from pulp mill and harbor sites using multivariate- and index-based approaches. *Environmental Science and Technology* 36(9), 1869-1878.

Zhou, H.L., Wu, H.F., Liao, C.Y., Diao, X.P., Zhen, J.P., Chen, L.L. and Xue, Q.Z. (2010) Toxicology mechanism of the persistent organic pollutants (POPs) in fish through AhR pathway. *Toxicology Mechanisms and Methods* 20(6), 279-286.

Appendix A Supporting information for Chapter 2

Sediment samples were collected throughout Jackfish Bay (Moberly Bay, Jackfish Bay and Tunnel Bay) using various sampling techniques (Table A.1). Surface sediment samples were collected using two different collection methods. Samples labelled “surface mixed” were collected using an Ekman grab and sediment was mixed before distributing to sample containers for PCDD/F analysis. Samples labeled “surface core” were collected with an Ekman grab, followed by collection of a 5 cm depth core sample from the middle of the grab ensuring no mixing. The core was then sectioned into 1 cm intervals and the top 2 cm were analysed for PCDD/F analysis. Core (i.e., “core (deep)”) samples were collected using a gravity corer as described in Section 2.3.3 (Chapter 2) and ranged in depth from approximately 20–30 cm. Core chronologies were developed by the University of Waterloo WATER lab (Hall lab) for each core and the year was reported at mid-depth for each 1 cm section (Tables A.2, A.3, A.4).

Table A.1 Sediment sampling sites, sample ID, type of sample, GPS coordinates and distance from Blackbird Creek (BBC).

Site	Sample ID	Sample collection	Coordinates	Distance from BBC (km)
Blackbird Creek	105	Surface mixed	N 48°48'39.4" W 87°00'06.7"	0.06
	104	Surface mixed	N 48°48'38.1" W 87°00'06.1"	0.09
	106	Surface core	N 48°48'35.4" W 87°00'05.7"	0.17
	109	Surface core	N 48°48'34.0" W 87°00'05.7"	0.22
Moberly Bay	007	Surface core	N 48°48'27.4" W 87°00'05.7"	0.42
	006	Surface core	N 48°48'26.7" W 87°00'04.1"	0.45
	081	Core (deep)	N 48°48'22.0" W 87°00'00.0"	0.59
Jackfish Bay	043	Core (deep)	N 48°47'28.5" W 86°59'23.9"	2.37
	045	Surface core	N 48°47'27.5" W 86°59'22.8"	2.44
	101	Surface core	N 48°47'10.2" W 86°59'23.9"	2.94
Tunnel Bay	067	Surface core	N 48°48'45.8" W 86°57'38.1"	3.06
	111	Surface core	N 48°48'41.6" W 86°57'34.9"	3.11
Santoy Bay	042	Core (deep)	N 48°45'42.0" W 86°54'36.4"	8.71
	035	Surface core	N 48°45'43.8" W 86°54'32.2"	8.73
	039	Surface core	N 48°45'43.2" W 86°54'32.6"	8.74
Mountain Bay	213	Surface core	N 48°54'36.9" W 87°48'15.0"	59.76
	210	Surface core	N 48°54'36.9" W 87°48'07.7"	59.90

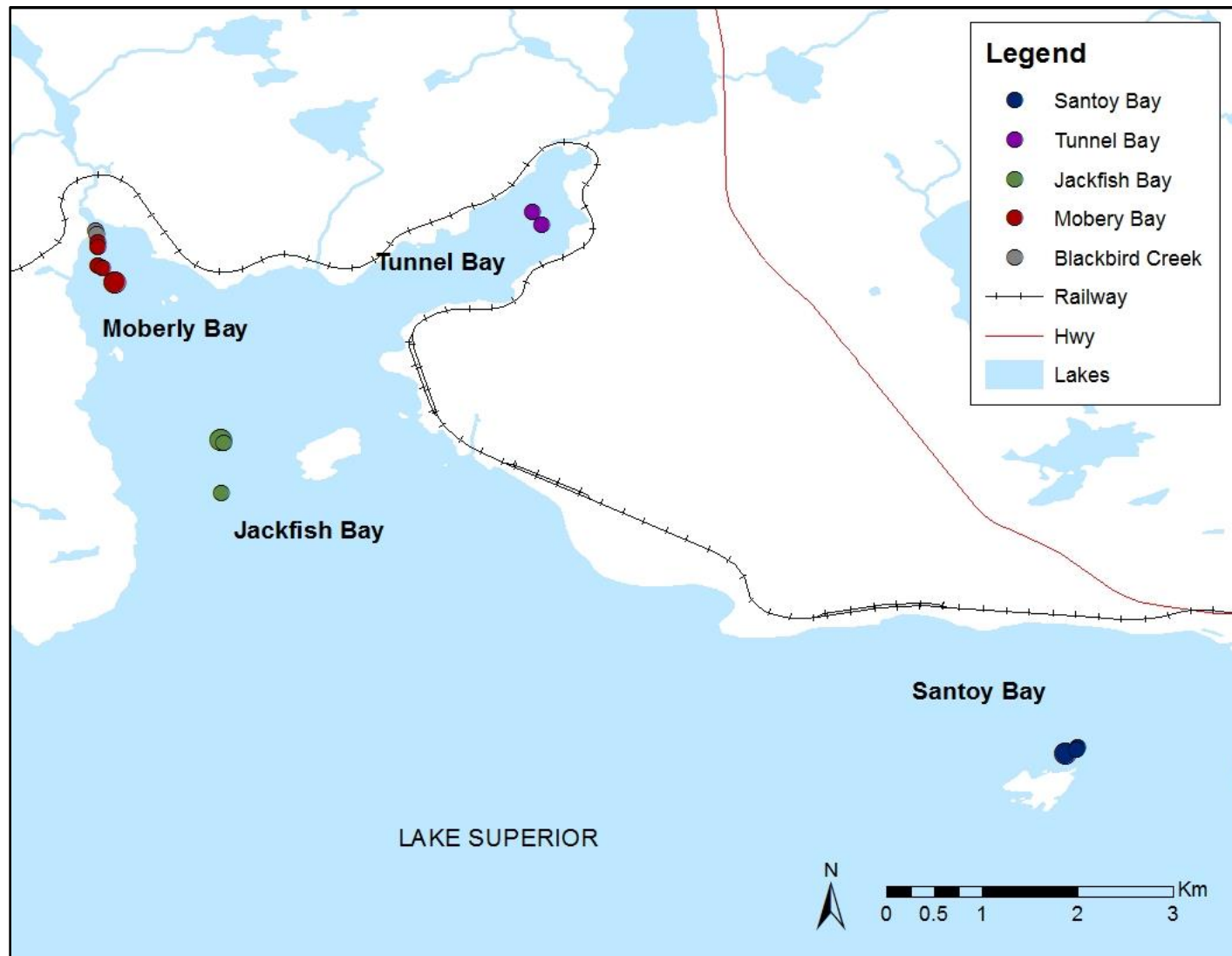


Figure A.1 Sampling sites for surface sediment (small circles) and sediment cores (large circles) collected throughout Jackfish Bay and Santoy Bay.

Moberly Bay Chronology Report

The sediment core collected from the depositional area in Moberly Bay (N48°48'22.0 W87°00'00.0") spanned 21 cm in length. Sixteen 1 cm sections were analysed for ^{210}Pb , ^{214}Bi , ^{214}Pb , ^{137}Cs , ^{40}K , ^{238}U equivalent, and ^{232}Th equivalent activity using gamma ray spectrometry to develop the ^{210}Pb dating core chronologies using the Constant Rate of Supply model (CRS). At a depth of 10 cm (and deeper) the unsupported ^{210}Pb inventory began to asymptotically approach zero and was no longer directly measureable; below 10 cm unsupported ^{210}Pb activity was estimated using the exponential regression function ($R^2 = 96\%$) within Sigma Plot. Peak ^{137}Cs activity was measured in the 6–7 cm section of this core and is expected to represent ~1963 when peak atmospheric ^{137}Cs weapons fallout occurred in North America. This date, however, is not consistent with the CRS model. A rapid decline in ^{137}Cs activity and % organic matter of sediment below this level suggest changes to the sediment supply and composition during this time period.

Three different age models (CRS, CF:CS and ^{137}Cs age model) estimated the age of the core as far back as the mid-1600s, however, dates prior to 1919 were determined using linear extrapolation which assumes a constant rate of sedimentation. Large fluctuations in % organic matter were observed throughout the core, indicating that the sedimentation rate over time has likely not been constant. Further, the ^{210}Pb fallout measured for this core was only approximately 40% of the predicted annual fallout based on the latitude. This provides evidence that this area may not represent a pure deposition zone and some fine sediment may be transported to a further/deeper, more stable depositional zone.

The following figures were provided by Johan A. Wiklund (Hall lab) to represent the activity profile (Fig. A.2), CRS- and CF:CS modeled age-depth relationships (Fig. A.3), ^{137}Cs activity vs. CRS modeled age (Fig. A.4) and dry mass sedimentation rate (Fig. A.5) for the core collected from Moberly Bay.

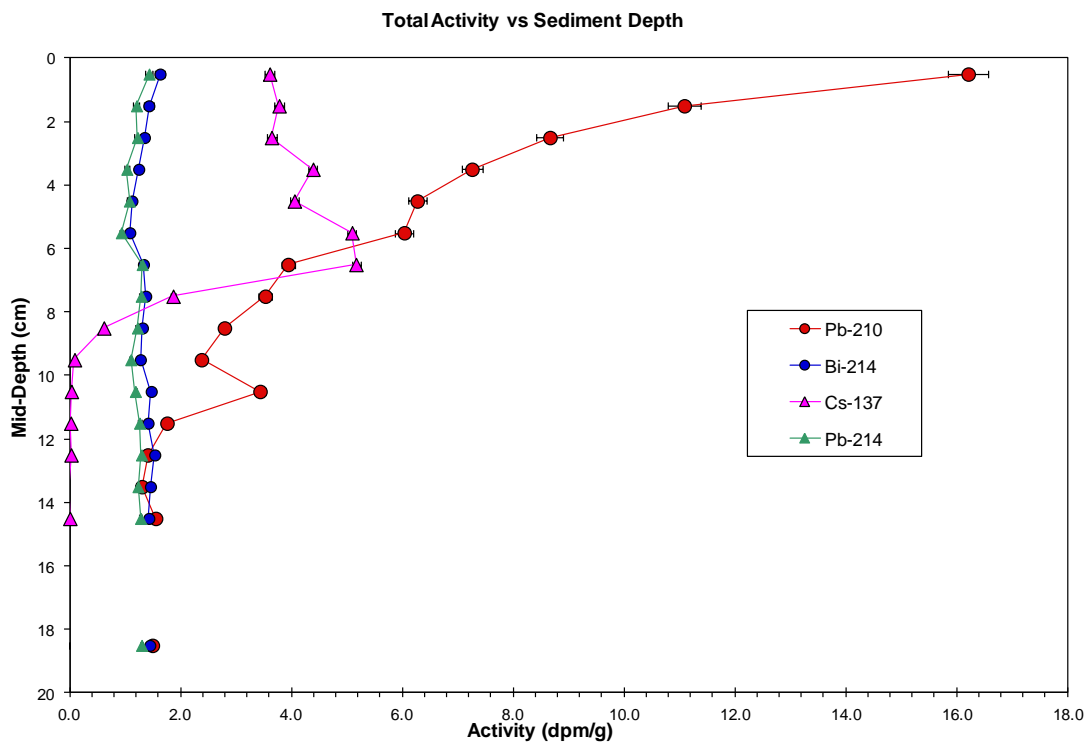


Figure A.2 Activity profile of sediment core collected from Moberly Bay.

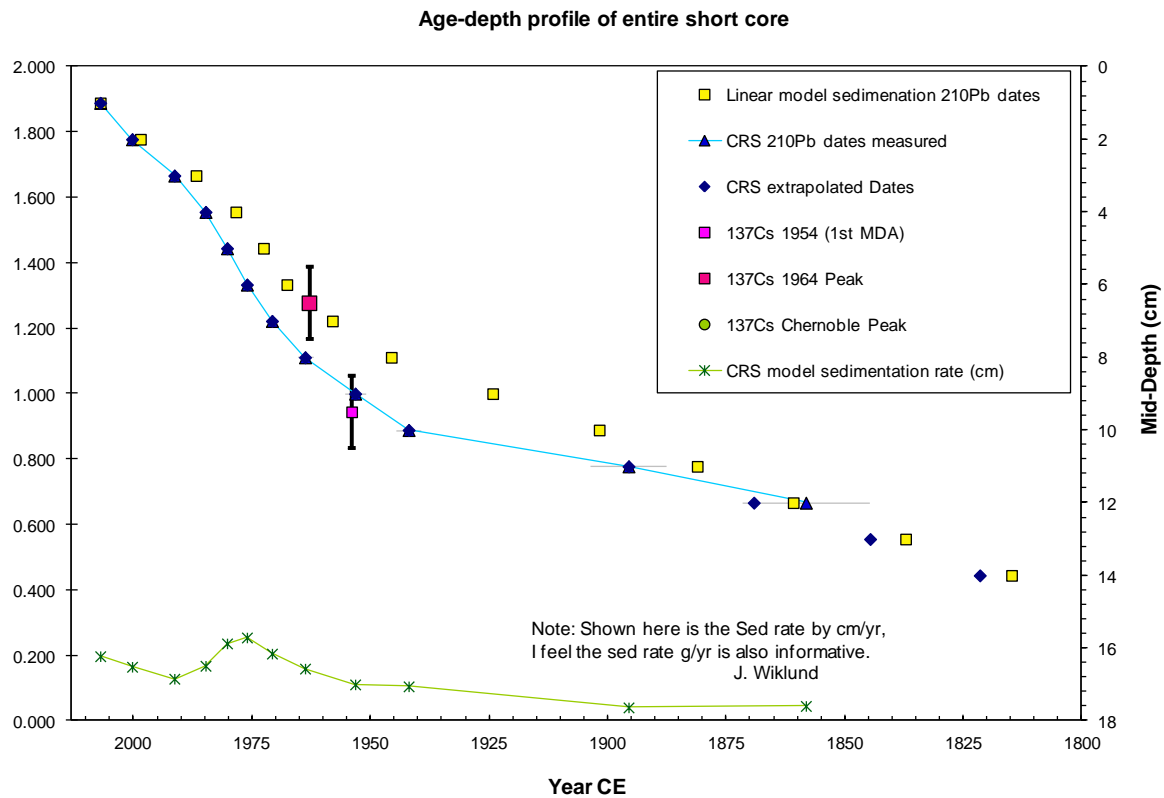


Figure A.3 Age depth profiles (CRS and CF:CS models) for Moberly Bay sediment core.

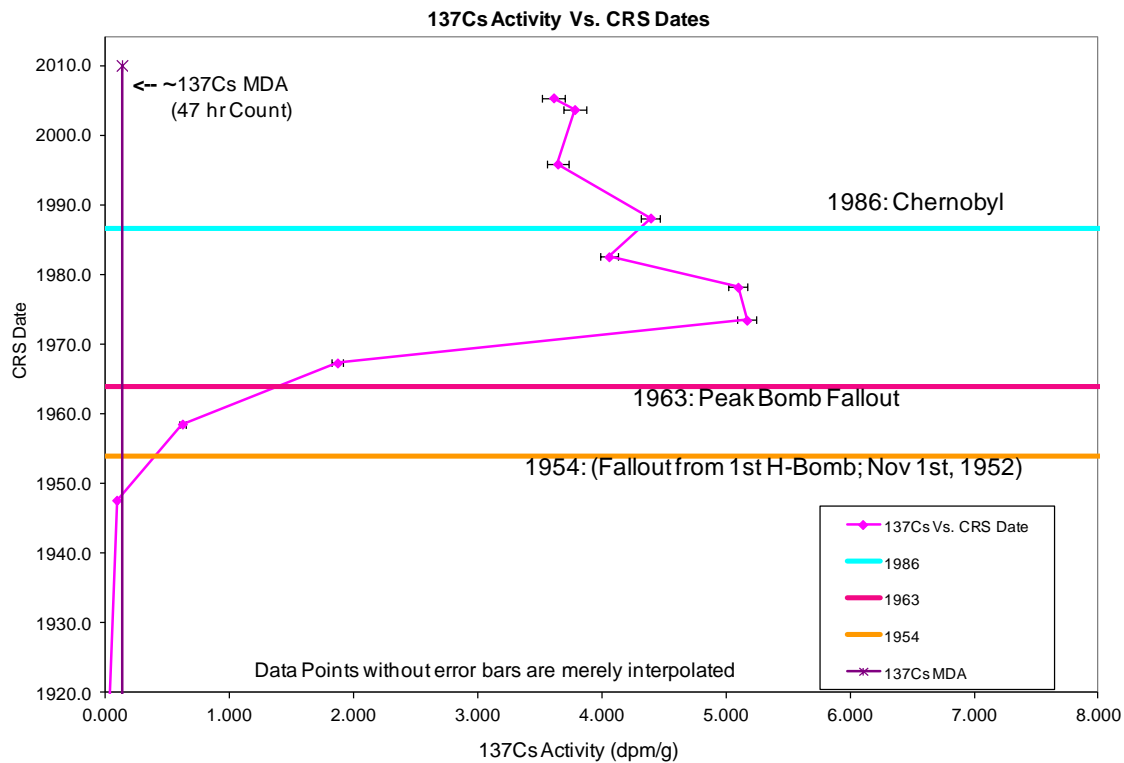


Figure A.4 ^{137}Cs activity vs. CRS age for Moberly Bay sediment core.

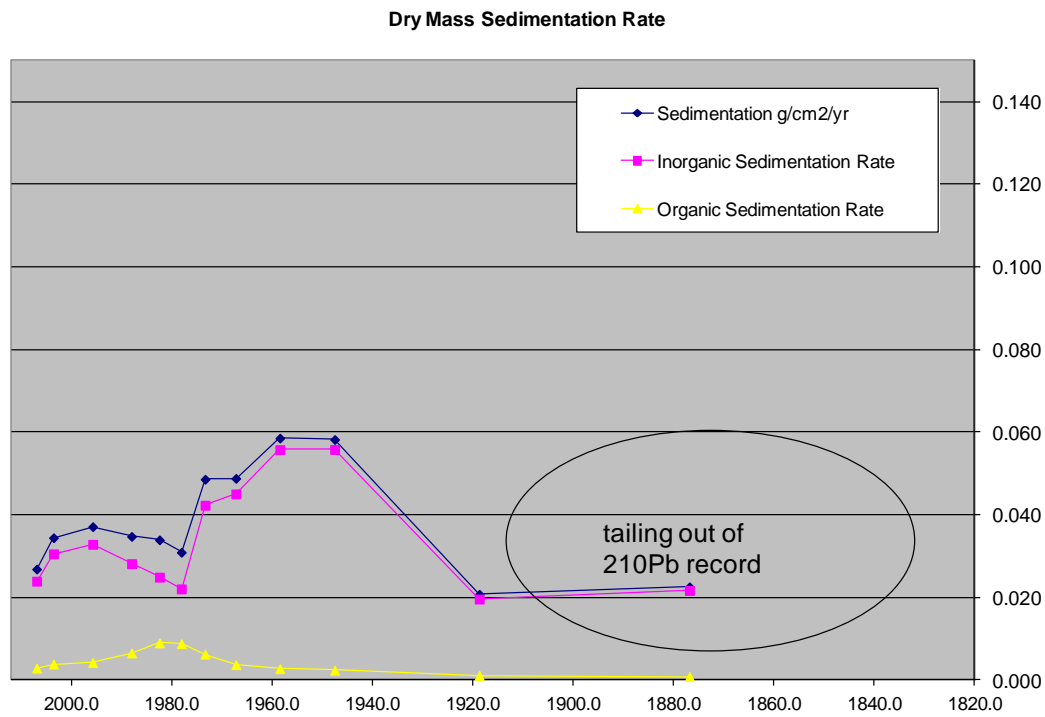


Figure A.5 CRS determined dry mass sedimentation rates vs. CRS age for Moberly Bay sediment core.

Table A.2 PCDD/F concentrations as dry weight ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and organic matter (OM) normalized ($\text{pg}\cdot\text{g}^{-1}$ OM) TEQ for a dated sediment core collected in Moberly Bay.

	1886	1919	1948	1959	1967	1973	1978	1983	1988	1996	2004	2005
2,3,7,8-TCDD	0.51	3.33	2.99	4.98	16.7	22.9	31.3	45.4	42.2	21.4	4.57	3.63
1,2,3,7,8-PeCDD	0.19	0.66	0.39	0.54	0.79	1.00	2.65	4.48	3.75	1.81	0.53	0.50
1,2,3,4,7,8-HxCDD	0.10	0.45	0.27	0.45	0.60	1.17	2.29	2.16	1.90	1.00	0.41	0.39
1,2,3,6,7,8-HxCDD	0.30	1.33	0.75	1.11	1.53	3.33	6.44	4.94	4.01	2.25	0.96	0.85
1,2,3,7,8,9-HxCDD	0.34	1.57	0.82	1.31	1.98	3.37	6.82	6.01	4.74	2.68	1.15	1.35
1,2,3,4,6,7,8-HpCDD	2.00	9.65	6.50	12.3	16.2	66.8	130	80.5	65.0	36.8	16.2	16.2
OCDD	8.62	52.7	37.3	62.3	74.8	338	629	444	344	226	103	103
2,3,7,8-TCDF	2.74	18.4	16.9	41.9	225	288	422	808	777	374	74.5	59.0
1,2,3,7,8-PeCDF	0.17	0.54	0.36	0.64	1.65	1.99	6.93	16.6	14.9	10.8	2.8	1.9
2,3,4,7,8-PeCDF	0.18	0.87	0.56	1.08	2.88	3.73	9.99	25.3	26.6	16.0	3.6	2.9
1,2,3,4,7,8-HxCDF	0.22	0.91	0.56	0.80	1.61	2.53	3.74	4.43	4.03	3.11	1.13	1.03
1,2,3,6,7,8-HxCDF	0.21	0.66	0.36	0.50	0.81	1.19	1.64	1.45	1.35	1.03	0.48	0.46
1,2,3,7,8,9-HxCDF	ND	ND	ND	0.06	0.08	0.10	0.20	0.27	0.26	0.18	0.11	ND
2,3,4,6,7,8-HxCDF	0.13	0.57	0.33	0.44	0.68	1.05	1.33	1.20	1.10	0.65	0.37	0.34
1,2,3,4,6,7,8-HpCDF	1.43	4.92	2.75	5.03	6.81	30.2	45.2	21.6	18.7	10.4	5.3	4.8
OCDF	0.41	2.68	1.82	10.3	9.70	105	168	83.2	65.5	36.8	16.0	10.8
% OM	3.95	5.51	4.15	4.77	7.69	12.9	28.6	26.6	18.9	11.5	11.3	10.9
TEQ^b	1.07	5.92	4.85	8.69	31.0	41.8	63.0	106	101	51.7	11.3	9.12
TEQ^b_(OM)	27.1	108	117	182	403	323	220	399	533	448	99.64	84.0

^b TEQ calculated using fish TEFs reported by Van den Berg *et al.* (1998)

Table A.3 PCDD/F concentrations as dry weight ($\text{pg}\cdot\text{g}^{-1}$ dry wt.) and organic matter (OM) normalized ($\text{pg}\cdot\text{g}^{-1}$ OM) TEQ for a dated sediment core collected in Santoy Bay.

	1914	1935	1965	1988	2003
2,3,7,8-TCDD	0.11	0.12	0.43	0.54	0.33
1,2,3,7,8-PeCDD	0.13	0.21	0.85	0.91	0.73
1,2,3,4,7,8-HxCDD	0.07	0.18	0.75	0.95	0.69
1,2,3,6,7,8-HxCDD	0.26	0.50	1.79	1.97	1.42
1,2,3,7,8,9-HxCDD	0.22	0.68	2.25	2.77	2.17
1,2,3,4,6,7,8-HpCDD	1.29	4.12	18.5	25.0	17.5
OCDD	4.34	21.5	90.9	113	72.0
2,3,7,8-TCDF	0.20	0.57	2.82	4.35	2.58
1,2,3,7,8-PeCDF	0.11	0.19	0.66	0.92	0.63
2,3,4,7,8-PeCDF	0.09	0.22	0.79	1.07	0.71
1,2,3,4,7,8-HxCDF	0.17	0.30	1.13	1.52	1.02
1,2,3,6,7,8-HxCDF	0.14	0.26	0.80	0.98	0.71
1,2,3,7,8,9-HxCDF	2.47E-03	4.39E-03	0.08	0.09	0.07
2,3,4,6,7,8-HxCDF	0.09	0.21	0.73	0.89	0.61
1,2,3,4,6,7,8-HpCDF	0.97	2.05	5.82	6.40	9.98
OCDF	0.22	1.29	4.30	5.60	5.46
% OM	3.59	5.48	8.24	10.6	7.63
TEQ^b	1.55	10.8	25	35.9	18.2
TEQ^b_(OM)	43.1	197	304	339	238

^b TEQ calculated using fish TEFs reported by Van den Berg *et al.* (1998)

Table A. PCDD/F concentrations as dry weight (pg·g⁻¹ dry wt.) and organic matter (OM) normalized (pg·g⁻¹ OM) TEQ for a dated sediment core collected in Jackfish Bay near St. Patrick Island.

	1919	1948	1971	1990	2006
2,3,7,8-TCDD	0.52	4.71	11.4	15.4	7.37
1,2,3,7,8-PeCDD	0.24	1.15	1.91	2.28	1.37
1,2,3,4,7,8-HxCDD	0.19	0.98	1.58	1.58	1.14
1,2,3,6,7,8-HxCDD	0.49	2.58	3.74	3.27	2.24
1,2,3,7,8,9-HxCDD	0.45	3.27	4.98	4.61	3.20
1,2,3,4,6,7,8-HpCDD	3.25	28.0	52.0	44.9	30.5
OCDD	15.3	149	270	215	143
2,3,7,8-TCDF	6.14	54.5	147	233	108
1,2,3,7,8-PeCDF	0.43	1.39	3.39	5.68	3.78
2,3,4,7,8-PeCDF	0.41	1.98	4.97	9.19	5.29
1,2,3,4,7,8-HxCDF	0.50	1.93	2.75	2.89	2.10
1,2,3,6,7,8-HxCDF	0.40	1.26	1.66	1.57	1.15
1,2,3,7,8,9-HxCDF	2.45E-03	0.11	0.22	0.19	0.18
2,3,4,6,7,8-HxCDF	0.31	1.06	1.36	1.38	1.03
1,2,3,4,6,7,8-HpCDF	2.30	10.1	13.5	11.2	7.90
OCDF	1.92	12.5	26.6	17.4	11.1
% OM	2.87	3.68	3.71	34.4	10.1
TEQ^b	0.38	0.69	2.62	3.21	2.32
TEQ^b_(OM)	13.3	18.6	70.7	9.34	23.0

^b TEQ calculated using fish TEFs reported by Van den Berg *et al.* (1998)

Appendix B Supporting information for Chapter 3

To allow a direct comparison of toxic equivalence (TEQ) measured in white sucker collected downstream of Smooth Rock Falls in 1993 and 1995 by Servos et al. (1997), mammalian TEQ values were calculated using international toxic equivalency (I-TEF) values (Table B.1).

Table B.1 International toxic equivalency factors (I-TEF) of PCDD/F congeners for mammals reported by Kutz et al. (1990)

		Toxic equivalency factor (I-TEF)
	Congener	Mammals
Dioxins	2,3,7,8-TCDD	1
	1,2,3,7,8-PeCDD	0.5
	1,2,3,4,7,8-HxCDD	0.1
	1,2,3,6,7,8-HxCDD	0.1
	1,2,3,7,8,9-HxCDD	0.1
	1,2,3,4,6,7,8-HpCDD	0.01
	OCDD	0.001
Furans	2,3,7,8-TCDF	0.1
	1,2,3,7,8-PeCDF	0.05
	2,3,4,7,8-PeCDF	0.5
	1,2,3,4,7,8-HxCDF	0.1
	1,2,3,6,7,8-HxCDF	0.1
	1,2,3,7,8,9-HxCDF	0.1
	2,3,4,6,7,8-HxCDF	0.1
	1,2,3,4,6,7,8-HpCDF	0.01
	OCDF	0.01